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27 Abstract Laboratory scale anaerobic digestion studies were carried out to determine the effect of high-shear mixing on the degradation of lipids. The studies showed that the intensity of mixing must be carefully tailored to the rate and type of feed if benefits are to be realized. Dog food, cottonseed oil, and motor oil were fed to the digesters daily as slug loads. When properly operated, the 15 replicate digesters gave reproducible results with good precision. High-shear mixing has little effect on degradation of dog food and was deleterious to lime-buffered solutions heavily loaded with cotton seed oil. When soda ash was used as a buffering agent, heavy loads of oil caused the digester to "go sour" regardless of the mixing system. Under some conditions homogenization led to serious foaming in the digestors. Kinetic data and chemical analysis of drawdown samples confirmed the hypothesis that homogenization or soda ash buffering can accelerate the hydrolysis of a heavy load of fat to a point where the saponification products over load the methane fermentation. Motor oil was not readily digested but did not appear to influence the digestion of cottonseed oil or dog food. Other observations were: (1) more rapid gasification occurs as the feed rate increases; (2) lime and soda ash cannot be used interchangeably as buffers; and (3) feed COD cannot be used to predict methane yield. (Ellis-Texas)

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OPTIMIZING LIPID BIOSTABILIZATION

by

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for the

FEDERAL WATER QUALITY ADMINISTRATION

DEPARTMENT OF THE INTERIOR

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ABSTRACT

Laboratory-scale anaerobic digestion studies were carried out to determine the effect of high-shear mixing (homogenization) on the degradation of lipids. The studies showed that the intensity of mixing must be carefully tailored to the rate and type of feed if benefits are to be realized.

Dog food, cottonseed oil and motor oil were fed to the digesters daily as slug loads. When properly operated, the 15 replicates digesters gave reproducible results with good precision.

High-shear mixing has little effect on degradation of dog food and was deleterious to lime-buffered solutions heavily loaded with cottonseed oil. When soda ash was used as a buffering agent, heavy loads of oil caused the digester to "go sour" regardless of mixing system, although homogenization gave somewhat better results. Under some conditions, homogenization led to serious foaming in the digestors. Kinetic data and chemical analysis of drawdown samples confirmed the hypothesis that homogenization or soda ash buffering can accelerate the hydrolysis of a heavy load of fat to a point where the saponification products overload the methane fermentation or affect it by surface effects. Motor oil was not readily digested, but did not appear to influence the digestion of cottonseed oil or dog food.

Other observations were: more rapid gasification occurs as the feed rate is increased; lime and soda ash cannot be used interchangeably as buffers; feed COD cannot be used to predict methane yield; and the temperature response of a system greatly exceeds $Q_{10} = 2$.

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INTRODUCTION

Wastewater must be renovated before it is returned to the environment or reused. At present, domestic wastewater is processed in the United States in order to remove pathogens and reduce the organic matter which, in serving as growth media for common soil and aquatic bacteria, would deplete the receiving stream of dissolved oxygen. The two unit processes of biosorption and settling are employed for this purpose. Settled organic matter consists of raw wastes as well as the flocculated bacteria that have adsorbed and absorbed the non-settleable organic matter in the stream. This settled organic matter is highly putrescible, and it must be stabilized before it is spread on agricultural land, used as landfill, or disposed of at sea.

Sludge can be stabilized by chemical oxidation in the processes of incineration or wet oxidation. It can also be stabilized by microbiological oxidation and by the process of methane fermentation (or anaerobic digestion). This latter process has been included in the design of most of the larger plants that have been constructed in the United States during the past several decades.[1] Digestion has many attractive features, but the trade-offs are such that abandonment of this process in favor of oxidation processes is gaining favor. Table I presents the advantages and disadvantages of anaerobic digestion.

The wide variety of natural substances of biological origin that are insoluble in water and soluble in such non-polar solvents as ether, chloroform, and benzene are classified by the technologist as "lipids" or "lipides," which is derived from the Greek λιπος for fat. "Grease" is a classification that is designated by the feel of the substance and the ability of the material to make transluscent paper transparent. The term "fat" implies a biological source, but a chemist would restrict the term to describe the variety of liquids and solids that are formed naturally by the esterification of long chain carboxylic acids and glycerol.

Loehr and Kukar[2] have shown that the complete spectrum of natural lipids is to be found in wastewater. Hunter and Heukelekian[3] place the quantity of grease in Highland Park, N. J., sewage at 25%. A variety of industrial wastes, especially from packing houses, dairies, and canning plants, would increase the level of fat, while petroleum products, especially automotive motor drain oil and shop floor scrubbings, would contribute a nonglyceride fraction.

TABLE I

ADVANTAGES AND DISADVANTAGES IN ANAEROBIC DIGESTION OF MUNICIPAL WASTE SLUDGES

Advantages	Disadvantages
Approximately 2/3 of the BOD in wastewater stream can be processed without cost of aeration	Equipment is more expensive than aeration equipment
Can process hydrophilic sludges of low solids content	Malodor from H_2S and other anaerobic metabolities
Digested sludge more filterable than fresh sludge	Process variables of pH, feed rate, N&P nutrition, temperature and inorganic concentration must be carefully controlled
Low ORP inhibits corrosion	Air-oxidized H_2S is highly corrosive
Some aerobically bioresistant materials are digested	Difficult to initiate fermentation
Produced methane can provide power for plant	Grease is concentrated in digesters as scum layers
	Oxygen is lethal to process
	Produces a strong supernatant liquor which constitutes a heavy organic and nutrient loading to main biological treatment process

A biochemical analysis of lipid digestion anticipates two sources of difficulty. First, normally occurring wild bacteria will not be able to metabolize certain synthetic molecular structures and some petroleum derivatives. There is no definitive study in the sanitary engineering literature on the relative rates of degradation of the various molecular species of hydrocarbons although Davis[4] presents sufficient evidence to establish that different rates do indeed exist. Some of Davis' own research[5] indicates that the normal paraffins are more rapidly oxidized by some aerobic microorganisms. Beerstecher,[6] on the other hand, states that the branched chain hydrocarbons are more susceptible to oxidation.

Jeris and McCarty[7] have shown that microbial mechanisms are available in a methane fermenter to convert palmitic acid and water to methane and carbon dioxide. The same order of thermodynamic potential exists for decane:

$$CH_3 - (CH_2)_8 - CH_3 + 6H_2O \longrightarrow 7CH_4 + 3CO_2 \quad (\Delta F = -37 \text{ kcal/mole})$$

Davis[4] cites an abundance of evidence that the sulfate-reducing bacteria can oxidize hydrocarbons. Anaerobic breakdown of hydrocarbons by other bacteria has not been investigated, except for a report by Chouteau et al.[8] that Pseudomonas aeruginosa could dehydrogenate n-heptane.

The second criterion for lipid digestion is the actual contact of the microorganism and the surface of the lipid. While in the metabolism of soluble materials, the rate of nutrient utilization is a function of the concentration of nutrient, the rate of metabolism in a heterogeneous system of lipid-water-bacteria is related to the actual interfacial area rather than bulk amounts of material. Desnuelle[9] has shown that surface area is rate-limiting in the lipase-catalyzed hydrolysis of triglycerides. This concept is easily extrapolated to the problem of scum-layer formation in a sludge digester. Lipids, even though degradable, separate and float to the top of the digester tank, forming a scum layer. Once this material has separated, it is unavailable as a medium for bacterial growth unless mechanical energy is introduced to redisperse the lipids in the water. McKinney[10] has recognized surface area as a possible rate-limiting factor and suggested a high-speed dispersing nozzle in the sludge recycle system as a solution to the grease scum problem.

The research described in this report was designed to evaluate this redispersal of scum layers as the rate-limiting process in digestion of a typical lipid that is recognized as biodegradable. The usual anaerobic digestion system was modified to permit high-shear homogenization of any lipids that separated as a supernatant layer. The protocol for the experiments was designed to answer the following questions:

1. Does high-shear homogenization have any observable effect on an established digestion pattern where lipids are present only in very small concentrations?

2. Does high-shear homogenization have any effect on the digestion process when high concentrations of a biodegradable lipid (cottonseed oil) are present?

3. Does the presence of a non-biodegradable lipid (motor oil) influence the effect of high-shear homogenization on the biodegradation of a biodegradable lipid (cottonseed oil)?

The above questions have been answered by the following series of experiments:

Experiment I - Performance of 15 replicate laboratory digesters on a standard loading and mixing regimen of Purina Dog Chow. (Question 1)

Experiment II - Effect of high-shear homogenization on the digestion of Purina Dog Chow. (Question 1)

Experiment III - Effect of homogenization on limed digesters heavily loaded with cottonseed oil. (Question 2)

Experiment IV - Effect of homogenization on soda-ash-buffered digesters when dog food loading is augmented with cottonseed oil. (Question 2)

Experiment V - Effect of homogenization on soda-ash-buffered digesters when cottonseed oil gradually replaces Purina Dog Chow as a feed. (Question 2)

Experiment VI - Effect of motor oil on the digestion of Purina Dog Chow and cottonseed oil. (Question 3)

The data from these studies also have been analyzed for any other possible information that might be useful for improving the anaerobic digestion of wastes.

EXPERIMENT I

PERFORMANCE OF DIGESTERS ON STANDARD LOADING AND MIXING REGIMEN

The data for the baseline studies must be derived from several different time periods. The overall experimental design was such that the digesters were "fed" to a level that produced failure. Thus, after each experiment that involved an overload "feed," there was a period of re-adaptation or re-acclimation to establish the bacterial population. This was followed by a period of several days' operation at a constant feed level.

Initially the digesters were filled to the 1 gal. (2,785 ml) level. The daily drawdown was 175 ml; the detention time, 21.6 days. The digesters were inoculated with screened digester sludge and fed at a rate of 1.32 g/ℓ/day of dog food for 17 days. The feed level was then increased incrementally to 3.96 g/ℓ/day. During the period 10-9-68 to 10-15-68, all 15 digesters were stirred by head gas recirculation only. This period can be compared to similar "blank" periods of 11-5-68 to 11-11-68, 11-21-68 to 12-5-68, and 3-9-69 to 3-14-69. The average gas production of the 15 digesters during the period 10-9-68 to 10-15-68 is plotted as Curve B in Figure 2, p. 10. This curve is quite linear.

The data from each of the four "blank" periods are presented in Table II. The most significant information to be derived from the data are the variability of the digesters in each period. This is presented as the standard deviation divided by the average. Thus the four periods can be compared. The value of 31.4% for the standard deviation for the period 10-9-68 to 10-15-68 is well within the average for microbiological systems while the other three sets of data show that the variability during these "blank" periods was very low for such a system.[11] One approach to the overall variability of the system is to average the four values of the standard deviations after weighting for the time period. This overall standard deviation was 12.4%. During the entire period of 10-24-68 to 1-7-69, the average gas production was 277 ℓ with a standard deviation of only 6.6%. The literature does not have comparable values because most previous studies with anaerobic digesters have been performed with a single unit.

During the nine-day period, 11-12-68 to 11-20-68, inclusive, the digesters were operated without head gas recirculation or liquid recirculation. The digesters were stirred only by occasional shaking. During this period, the 15 digesters produced an average of 33.75 ℓ of gas from a loading rate of 3.96 g/ℓ/day. The gas yield was 0.250 ℓ/g feed, but the

TABLE II

PERFORMANCE OF DIGESTERS DURING "BLANK" PERIODS

Digester	Cumulative Gas Production in Liters			
	10-9-68 to 10-15-68 (6 days)	11-5-68 to 11-11-68 (6 days)	11-21-68 to 12-5-68 (15 days)	3-9-69 to 3-14-69 (5 days)
1A	17.55	30.52	66.54	16.88
1B	30.70	30.89	65.07	17.34
1C	22.74	29.72	67.32	19.42
2A	29.76	29.81	70.09	16.51
2B	30.73	30.63	72.05	18.58
2C	45.45	30.82	73.11	19.73
3A	22.82	25.30	58.94	17.58
3B	15.93	28.86	70.48	17.71
3C	19.11	30.10	67.37	17.94
4A	30.00	28.14	57.73	17.85
4B	39.14	27.02	62.64	16.97
4C	30.01	28.34	65.29	15.79
5A	45.51	23.36	52.44	16.22
5B	39.64	26.99	63.19	16.29
5C	28.56	28.14	63.68	18.27
Average	29.85	28.58	65.06	17.54
Standard deviation as % of average	31.4	7.7	8.6	6.6
Feed rate g/ℓ/day	3.96	3.96	3.96	5.56
Gas yield ℓ/g feed	0.331	0.318	0.289	0.292
Average methane content as %	--	54	54	49

nine-day period includes one day during which the bath temperature dropped to 20°C. The decrease in gas yield of this period when compared with the preceding and succeeding periods does not appear to be significant.

The baseline level of feed was to have been established at 75% of the feed level that would cause one-third of the digesters to malfunction either by becoming sour or passing through the feedstock undigested. It was not possible to produce biochemical malfunction as the feed level of dog food could be raised to only 20 g/gal. before the liquid pumping system became clogged. This is comparable to a loading of 0.28 lb volatile solid/ft^3/day (4.45 g/ℓ/day).

The data from this build-up period, 12-9-68 to 12-16-68, are presented in Figure 1. When computer based curve fitting techniques were applied to these data by Dr. James J. Downs, a linear equation,

GAS PRODUCTION = -0.032 + 0.072 FEED RATE

was obtained that accounted for 52% of the variance in the data. The inclusion of the temperature data gave the equation:

GAS PRODUCTION = -23.3 + 0.023 FEED RATE + 4.1 ln T°K

that accounted for 78% of the variance. The negative constants are disturbing until it is realized that these data represent only a small portion of the full range of possible relationships that would have the shape of a typical growth curve. This analysis of these data leads to the inference that digestion rate per unit waste load would increase with loading rate at some levels of loading.

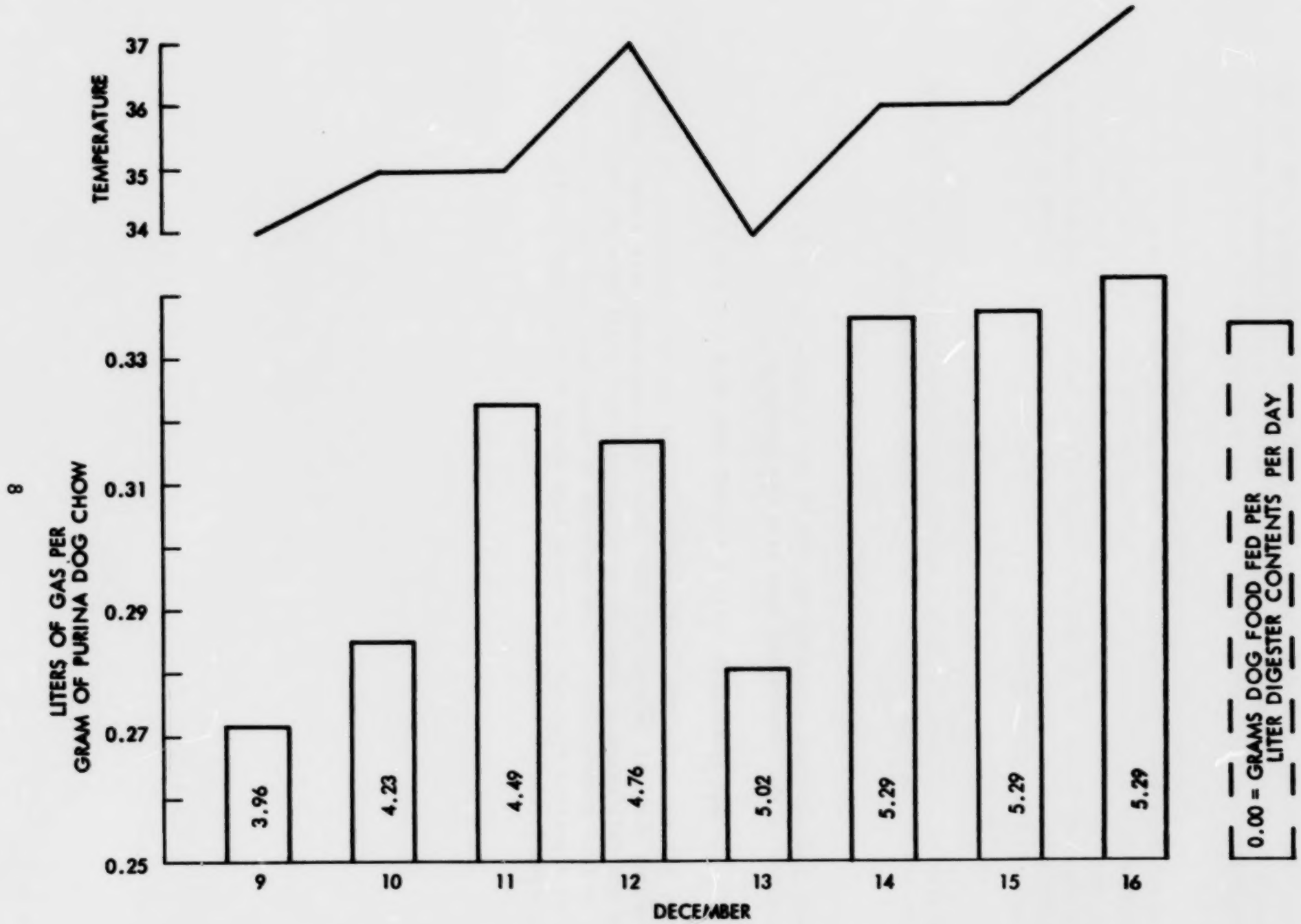

Figure 1 - Effect of Feed Rate on Gas Production

EXPERIMENT II

EFFECT OF HIGH-SHEAR HOMOGENIZATION ON THE DIGESTION OF PURINA DOG CHOW

The digesters were operated during the seven-day period, 10-16-68 to 10-23-68, with a constant dog food loading. The 15 digesters were divided into five groups of three:*

Group 1 - gas-stirred only;

Group 2 - Gas plus liquid stirring with spring-loaded ball valve operating at 50 psig;

Group 3 - Gas plus liquid stirring plus homogenization with relief valve set at 100 psig;

Group 4 - Gas plus liquid stirring plus homogenization with relief valve set at 300 psig; and

Group 5 - Gas plus liquid stirring with relief valve set at 900 psig.

The digesters were filled to 3,785 ml and were maintained on a daily feed of 15 g (3.96 g/ℓ/day), 0.23 lb volatile solids/ft^3/day. The detention time was 21.6 days. The pH was determined each day before feeding and the system was buffered with lime ($Ca(OH)_2$), and sodium bicarbonate ($NaHCO_3$) so that 1 g of lime was added with the feed for each 0.10 pH unit below 7.00 to a maximum of 5 g and 0.1 g of $NaHCO_3$ was added with the feed for each 0.10 pH unit below 6.50. Head gas was recycled at the rate of 100 ml/min. Liquid recirculation rate was 100 ml/hr.

The results of the experiment are presented in Figure 2. There is a greater variability in this group of data than in the data from the later studies. Part of this is the result of averaging three rather than five data. There appears, however, to be no significant effect in the digestion process due to the homogenization process. Group 2 had the greatest rate of gas production of the five groups but the rate is only a few percent greater than the previous six-day "blank" period. Group 3 is definitely lower in the early part of the study, but the line joining the data of the last four days has the same slope as lines 1, 4, and 5.

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* In later experiments, the digesters were divided into three groups of five. The coding of the digesters, however, as 1A, 1B, 1C, 2A, 2B, etc., was maintained throughout the entire research and has been kept for this report.

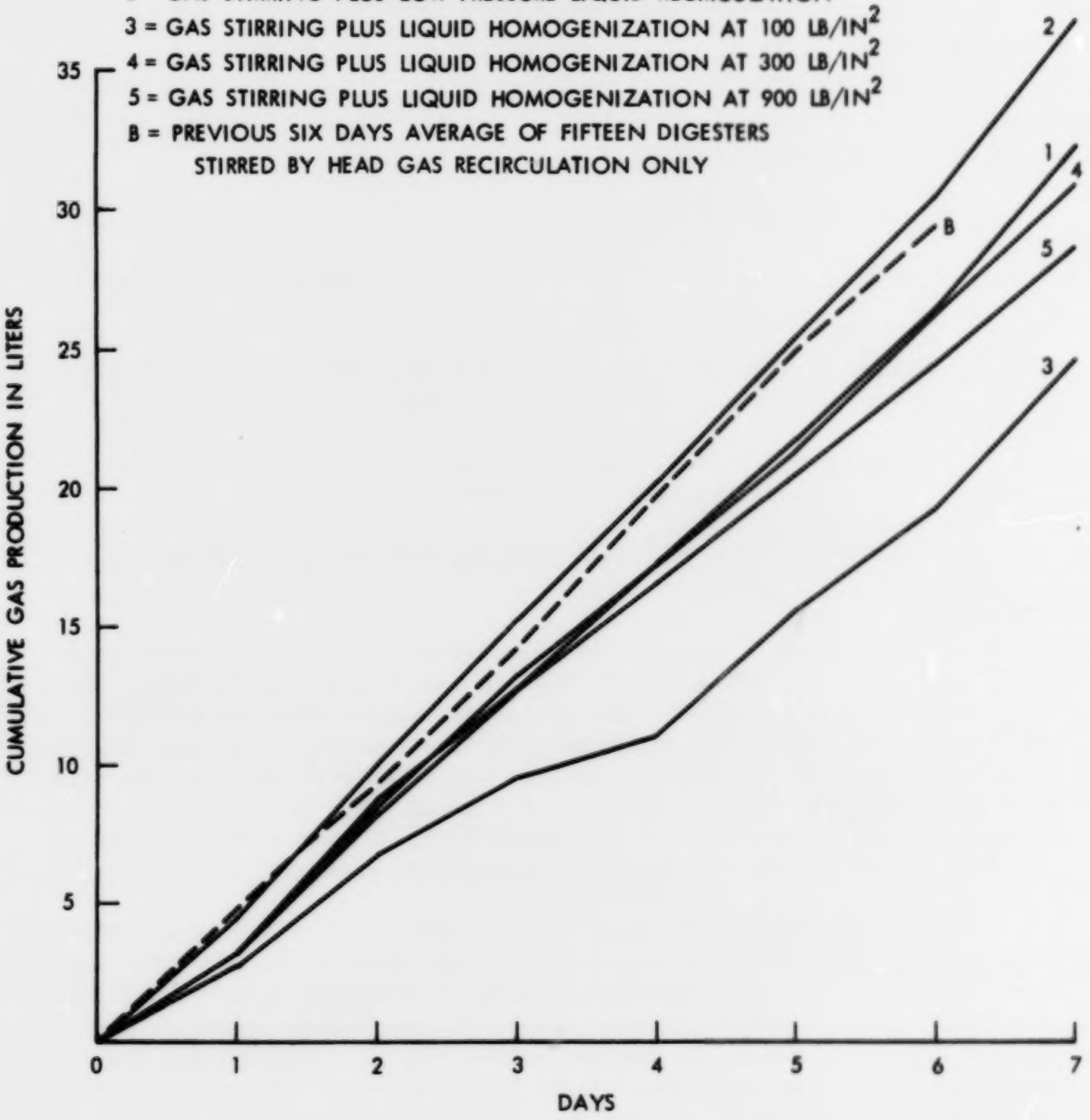

Figure 2 - Effect of Homogenization on Digestion

The experiment was discontinued after the seventh day because of foaming problems. The problem of foaming plagued the entire research program. This was the only pronounced effect on the digestion process due to high shear homogenization when lipid levels were low.

EXPERIMENT III

EFFECT OF HOMOGENIZATION ON LIMED DIGESTERS HEAVILY LOADED WITH COTTONSEED OIL

After Experiment II, the group of 15 digesters was divided into three groups of five:

Group A - Gas-stirred only;

Group B - Gas- and liquid-stirred with spring-loaded ball valve operating at 50 psig; and

Group C - Gas- and liquid-stirred plus homogenization with relief valve set at 300 psig.

Prior to Experiment III, the digesters were all functioning satisfactorily except for plugging of the diaphragm pumps when the feed level was raised to 20 g/gal./day. The digester contents had been pooled and redistributed. This slight exposure to the atmosphere had caused problems in the past, so the digesters were brought up to a feed level of 3.96 g/ℓ/day dog food over a period of a few days. The digesters were all performing satisfactorily and uniformly before the addition of cottonseed oil, as can be seen from the data presented in Table III.

TABLE III

PERFORMANCE OF DIGESTERS PRIOR TO ADDING COTTONSEED OIL

Date	Average Cumulative Gas Production in Liters			Remarks
	Group A	Group B	Group C	
26 December	3.84	4.21	4.18	3.96 g/ℓ/day Purina Dog Chow
27 December	4.32	5.36	4.74	Digester contents pooled
28 December	6.42	7.73	7.24	Raw sludge only added
29 December	8.52	9.63	9.29	1.32 g/ℓ/day Purina Dog Chow
30 December	11.75	12.02	12.39	2.64 g/ℓ/day Purina Dog Chow
31 December	15.70	14.81	15.38	3.96 g/ℓ/day Purina Dog Chow
1 January	21.00	18.75	18.95	3.96 g/ℓ/day Purina Dog Chow
2 January	25.60	22.30	24.32	3.96 g/ℓ/day Purina Dog Chow

In Experiment III, the digester volume was 3,785 ml; the detention time, 21.6 days. Head gas was recycled at the rate of 100 ml/min; liquid recirculation was at the rate of 100 ml/hr. Lime was added with the feed as a buffer to each individual digester at the rate of 1 g for each

0.10 pH unit below 7 until 1-11-69, after which 0.1 g of $NaHCO_3$ was added with the feed for each 0.10 pH unit below 7. The loading protocol (see Table IV) was such that the dog food was gradually replaced by cottonseed oil based on the approximate COD equivalence of 1 ml oil = 3 g dog food.

TABLE IV

PROTOCOL FOR DIGESTER LOADING IN EXPERIMENT III

Date	Dog Food (g COD/ℓ/day)	Cottonseed Oil (g COD/ℓ/day)	Total (g COD/ℓ/day)
3 January	3.73	0.70	4.43
4 January	0.00	0.70	0.70
5 January	2.49	0.70	3.19
6 January	2.49	0.70	3.19
7 January	3.73	0.70	4.43
8 January	3.73	0.70	4.43
9 January	3.73	1.40	6.13
10 January	2.99	2.10	5.09
11 January	2.24	2.80	5.04
12 January	2.24	2.80	5.04
13 January	2.24	2.80	5.04
14 January	1.49	3.50	4.99
15 January	1.49	3.50	4.99
16 January	1.49	3.50	4.99
17 January	1.49	3.50	4.99

The data on gas production are presented in Figure 3. These data, as well as analytical data presented in the Appendix, show that the three groups of digesters can be distinguished on every basis of comparison. Furthermore, Group A, which was the standard to which the other groups were to be compared, showed a unique behavior.

The following significant phenomena were observed during the 15-day period:

1. Group A produced more gas than Group B, which produced more gas than Group C.

2. The methane content of gas produced by Group A was higher than that produced by Group C as seen in Table V:

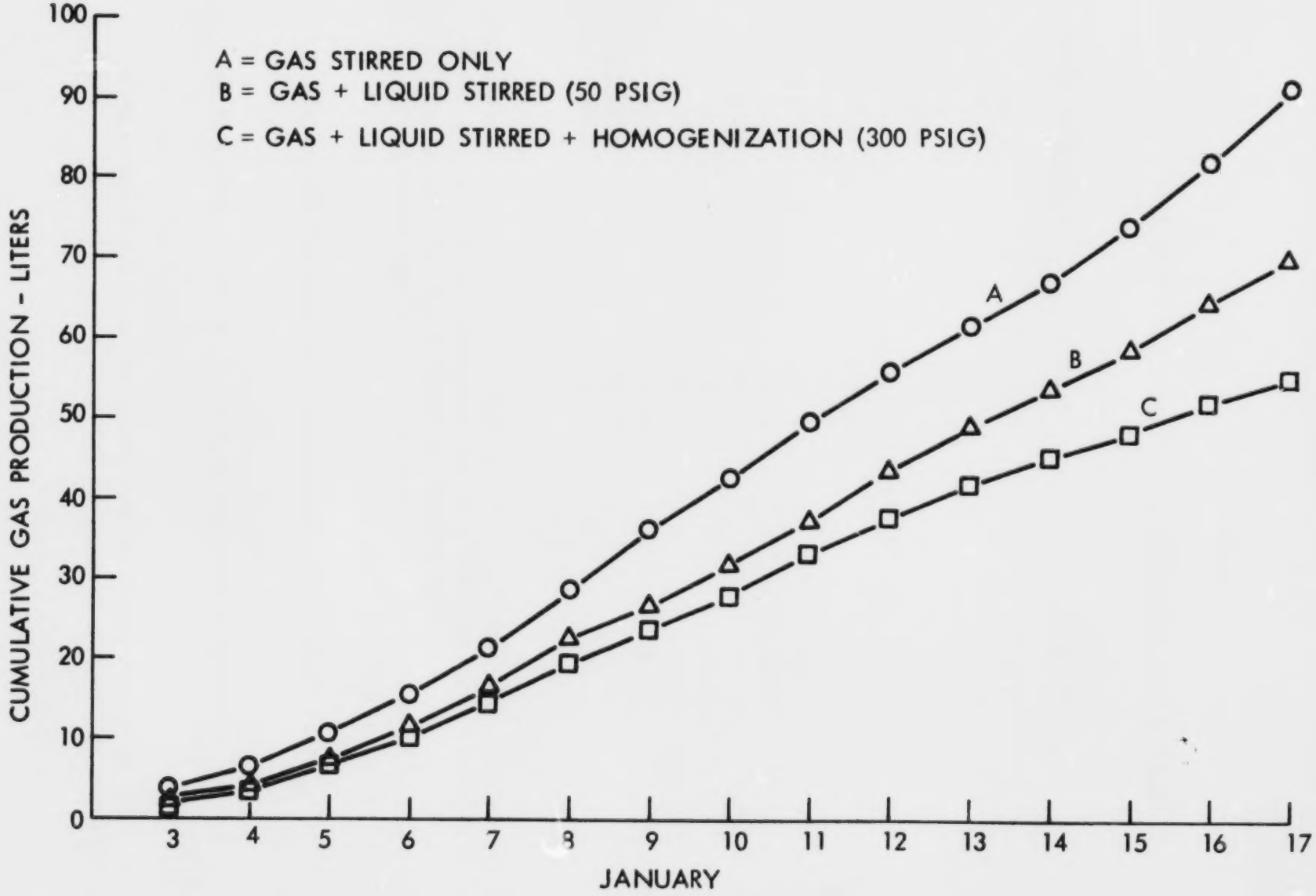

Figure 3 - Performance of Digesters Fed Dog Food and Cottonseed Oil Buffered with $Ca(OH)_2$

TABLE V

PERCENT METHANE IN DIGESTER GAS

	1-10-69	1-17-69
Group A	57.4	63.4
Group B	41.8	50.8
Group C	39.8	40.2

3. The data from the neutral lipid extraction, when examined for a single digester over a period of time, appear to reflect an adaptation of the bacterial population to the added cottonseed oil. There also seems to be a cyclic phenomenon in the individual lipid levels. When examined in relation to time, the average lipid levels in the various groups that are presented in Table VI imply the development of a bacterial flora more capable of hydrolyzing the neutral oil. In the case of Group C, however, the initial step of the breakdown of the oil to gas is too rapid, leading to a build-up of acid intermediates. Methane digestion is inhibited by the pH drop, and the slight increase in lipid levels of Group C that occurs between 1-13-69 and 1-16-69 may also be the result of pH drop affecting the hydrolyzing microorganisms.

TABLE VI

AVERAGE LIPID LEVEL IN EXPERIMENT III (g/ℓ)

Date	Group A	Group B	Group C	Total Added*
1-9-69	1.21	0.81	0.76	2.91
1-13-69	0.74	0.52	0.62	7.40
1-16-69	0.59	0.40	0.96	11.36

* Total added cottonseed oil from 1-3-69 to given date in grams per liter.

The soluble COD and MLVSS data also confirm the premise of differential adaptation. The MLVSS, which would measure both undigested dog food and bacterial mass, decreased as the dog food load decreased, but also decreased with the amount of shear. Soluble COD in Group A decreased from 1-7-69 to 1-14-69 as the dog food load was decreased but Group C maintained the same level. The decrease of MLVSS in the Group C digesters could be the consequence of the formation of calcium soaps, these coating the dog food particles and making them coalesce at the bottom of the digester.

The malfunctioning of the digesters in Groups B and C can be discerned as early as 1-8-69 when the pH values are examined. All pH's were above 6.80, but Group A had values all above 7.00. Since lime was administered to each individual digester at the rate of 1.0 g of $Ca(OH)_2$ for each pH unit below 7.0, the Group C digesters were most heavily limed. The lime maintained the pH in the normal operating range until 1-14-69 when all five digesters in this group had samples with pH readings 6.75 and below.

EXPERIMENT IV

EFFECT OF HOMOGENIZATION ON SODA-ASH BUFFERED DIGESTERS WHEN DOG FOOD LOADING IS AUGMENTED WITH COTTONSEED OIL

Prior to beginning this experiment, the liquid volume in all the digesters was reduced to 2,160 ml to allow the foam room for expansion. The detention time remained at 21.6 days. Head gas was recycled at the rate of 100 ml/min and liquid was recycled at the rate of 50 ml/hr. The contents of the Group A digesters were pooled and diluted with fresh sludge and this mixture was distributed evenly among all 15 digesters.

The digesters were then brought incrementally up to a daily dog food feed level of 5.55 g/ℓ (0.32 lb/ft^3). Soda ash, Na_2CO_3, was used to buffer at the rate of 0.2 g added with the feed to each digester for each 0.10 pH and below 7.00.

During the break-in period from 2-6-69 to 2-12-69, the 15 digesters produced 29.63 ℓ of gas with standard deviation = 2.42 or 11.5% of the mean. The dog food level was then maintained at 5.55 g/ℓ/day, while an additional 1 ml of cottonseed oil was added from 2-14-69 to 2-17-69 and 2 ml was added from 2-18-69 to 2-20-69. At the end of the experiment, the three groups of digesters could not be differentiated on the basis of cumulative gas production. These cumulative gas values, obtained with all pumps operating, were 23.71 ℓ, 23.98 ℓ, and 24.68 ℓ, respectively, for Groups A, B, and C. These data do not differentiate the groups. When daily gas production is plotted, as in Figure 4, some slight differences in the three groups may be discerned.

Table VII shows the results of lipid analyses during the period of Experiment IV. As in the case of Experiment III, where lime was used to buffer, the lipid level appears to be inversely related to the amount of shear.

TABLE VII

AVERAGE LIPID LEVEL IN EXPERIMENT VI (g/ℓ)

Date	Group A	Group B	Group C	Total Added*
2-10-69	1.12	0.81	0.69	0.00
2-13-69	0.89	0.75	0.56	0.00
2-17-69	2.15	1.60	1.22	1.69
2-20-69	2.65	1.83	1.84	4.22
2-24-69	1.46	1.22	0.705	4.22

* Total added cottonseed oil from 2-10-69 to given date.

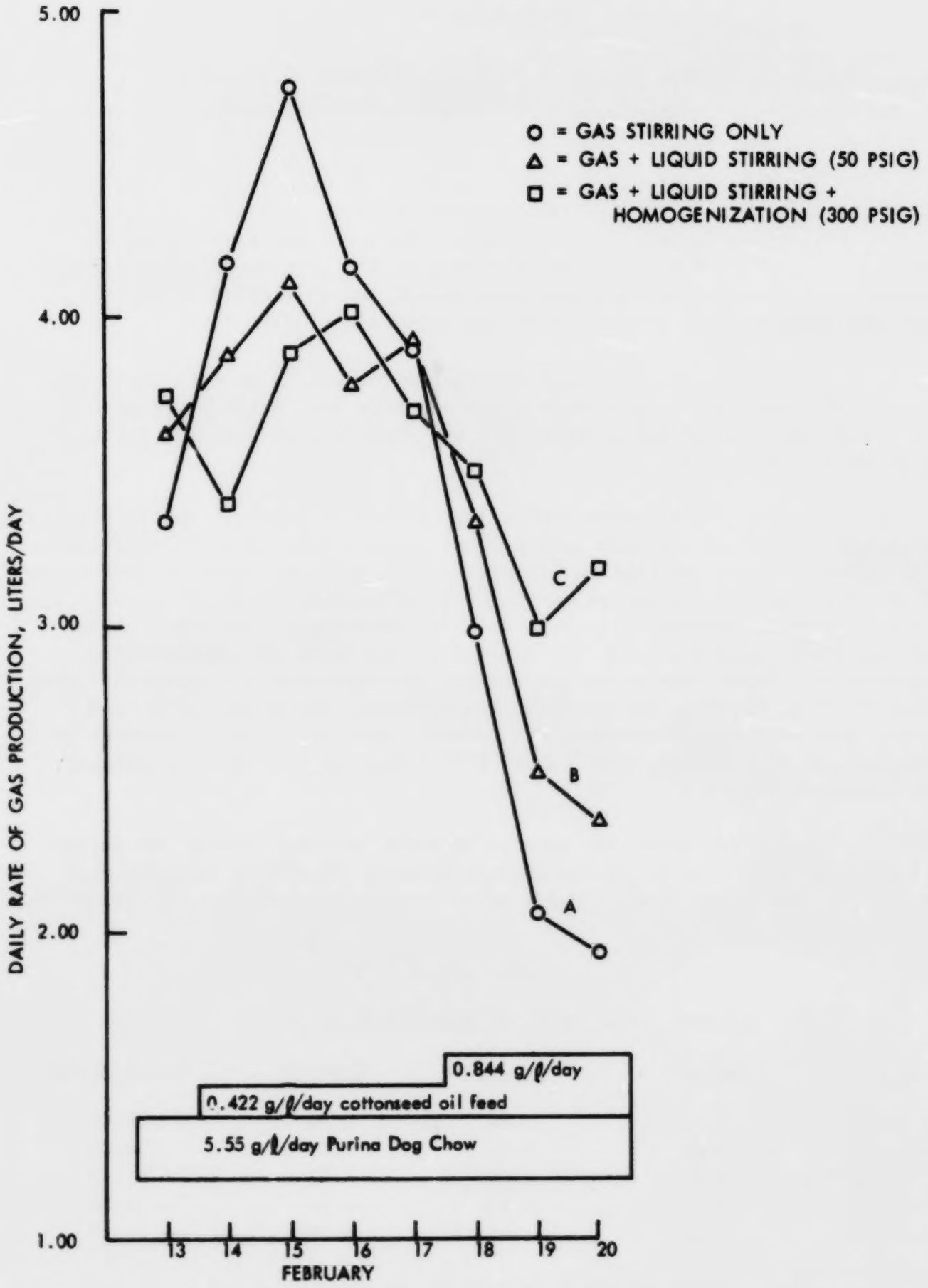

Figure 4 - Digester Performance with Soda-Ash Buffering

However, none of the three groups of digesters have adapted well to the slugs of cottonseed oil. The premise that the functioning of the hydrolyzing bacteria is affected by pH as well as the methanogenic bacteria is reinforced here. The Group A digesters, which initially appear to respond to the added oil, were the most rapid to fail. This can be seen in the volume of gas produced as shown in Figure 4 and in the methane content of the gas as well. On 2-14-69, all three groups of digesters were producing 53% methane while by 2-21-69 the average methane content of the gas produced by the three groups was 47, 51, and 52%, for Groups A, B, and C, respectively. The results of this experiment indicate that homogenization had a slight positive action in overcoming the malfunction caused by heavy oil loading accompanied by soda-ash buffering.

EXPERIMENT V

EFFECT OF HOMOGENIZATION ON SODA-ASH BUFFERED DIGESTERS AS COTTONSEED OIL GRADUALLY REPLACES PURINA DOG CHOW AS A FEED

This experiment was similar to Experiment IV except that the level of dog food loading was decreased while the level of cottonseed oil was increased. The volume of the liquid in the digesters was 2,160 ml; the detention time, 21.6 days. Soda ash was used to buffer the individual digesters at the rate of 0.2 g added with the feed for each 0.10 pH unit below 7.00. Protocol for loading as well as gas production and pH data are presented in Table VIII.

The major conclusion of this experiment is that heavy loads of cottonseed oil are deleterious to the performance of soda-ash buffered digesters. All three groups of digesters showed malfunctions as the percentage of the COD load that was due to cottonseed oil was increased. The three groups of digesters showed some differences in performance and, if rated by the usual criteria, the digesters subjected to gas and liquid stirring (Group B) gave a slightly better performance than the other two groups. The slight difference (4%) in the cumulative gas production between Groups B and C is probably not significant. If the difference in performance of the liquid stirred digesters was due to shear, it appears that the spring-loaded ball valve, operating at 50 psig, could provide adequate shear, if, indeed, the observed differences were due to shear.

The pH data from this experiment are presented in Figure 5. Groups B and C appear to sour a day later than Group A. The MLVSS data also appear to differentiate Group A from the other two groups as can be seen in Table IX. The soluble COD values for 4-1-69 that were 24.2, 17.0, and 15.8 g/ℓ for Groups A, B, and C, respectively, also appear to differentiate.

TABLE IX

MIXED LIQUOR VOLATILE SUSPENDED SOLIDS IN EXPERIMENT V (g/ℓ)

Date	Group A	Group B	Group C
3-18-69	22.1	23.1	17.8
3-25-69	14.2	16.2	12.5
4-1-69	16.0	16.1	13.5

TABLE VIII

EFFECT OF HOMOGENIZATION ON THE DIGESTION OF COTTONSEED OIL WHEN FEED RATE IS KEPT CONSTANT IN COD

Date	Feed Rate in Grams (COD/ℓ/day)		Cumulative Gas Production in Liters			Average pH in 15 Digesters	pH Range in 14 Digesters*
	Purina Dog Chow	Cottonseed Oil	Group A	Group B	Group C		
3-15-69	5.23	0	3.73	3.80	4.19	6.95	6.80-7.30
3-16-69	5.23	0	8.14	8.16	8.49	6.97	6.85-7.30
3-17-69	5.23	0	11.45	11.95	12.08	7.06	6.95-7.25
3-18-69	5.23	0	15.93	16.14	15.33	6.93	6.85-7.20
3-19-69	5.23	0	17.93	19.75	19.25	6.93	6.85-7.25
3-20-69	4.58	0.61	21.06	23.11	22.58	6.91	6.70-7.15
3-21-69	4.58	0.61	23.85	26.30	25.67	6.87	6.70-7.10
3-22-69	4.58	0.61	27.23	29.78	29.09	6.97	6.85-7.05
3-23-69	3.94	1.22	31.17	33.87	32.98	6.96	6.90-7.10
3-24-69	3.94	1.22	33.14	36.23	34.83	6.92	6.80-7.15
3-25-69	3.94	1.22	35.80	39.40	37.86	6.84	6.65-7.10
3-26-69	3.29	1.83	37.97	42.02	40.39	6.85	6.70-7.05
3-27-69	3.29	1.83	39.92	44.50	42.52	6.80	6.60-7.00
3-28-69	3.29	1.83	42.01	46.91	44.89	6.80	6.60-7.05
3-29-69	2.62	2.44	43.24	48.23	45.86	6.66	6.50-6.80
3-30-69	2.62	2.44	45.08	49.90	47.74	6.57	6.40-6.90
3-31-69	2.62	2.44	46.11	51.62	49.30	6.51	6.25-7.05
4-1-69	3.29	3.05	47.48	53.49	51.12	6.47	6.30-6.90
4-2-69	3.29	3.05	48.31	54.41	52.24	6.46	6.30-6.90
4-3-69	3.29	3.05	49.16	55.90	54.07	6.36	6.30-6.75

* Digester 1A was malfunctioning during most of this study, and its pH has not been included in the range.

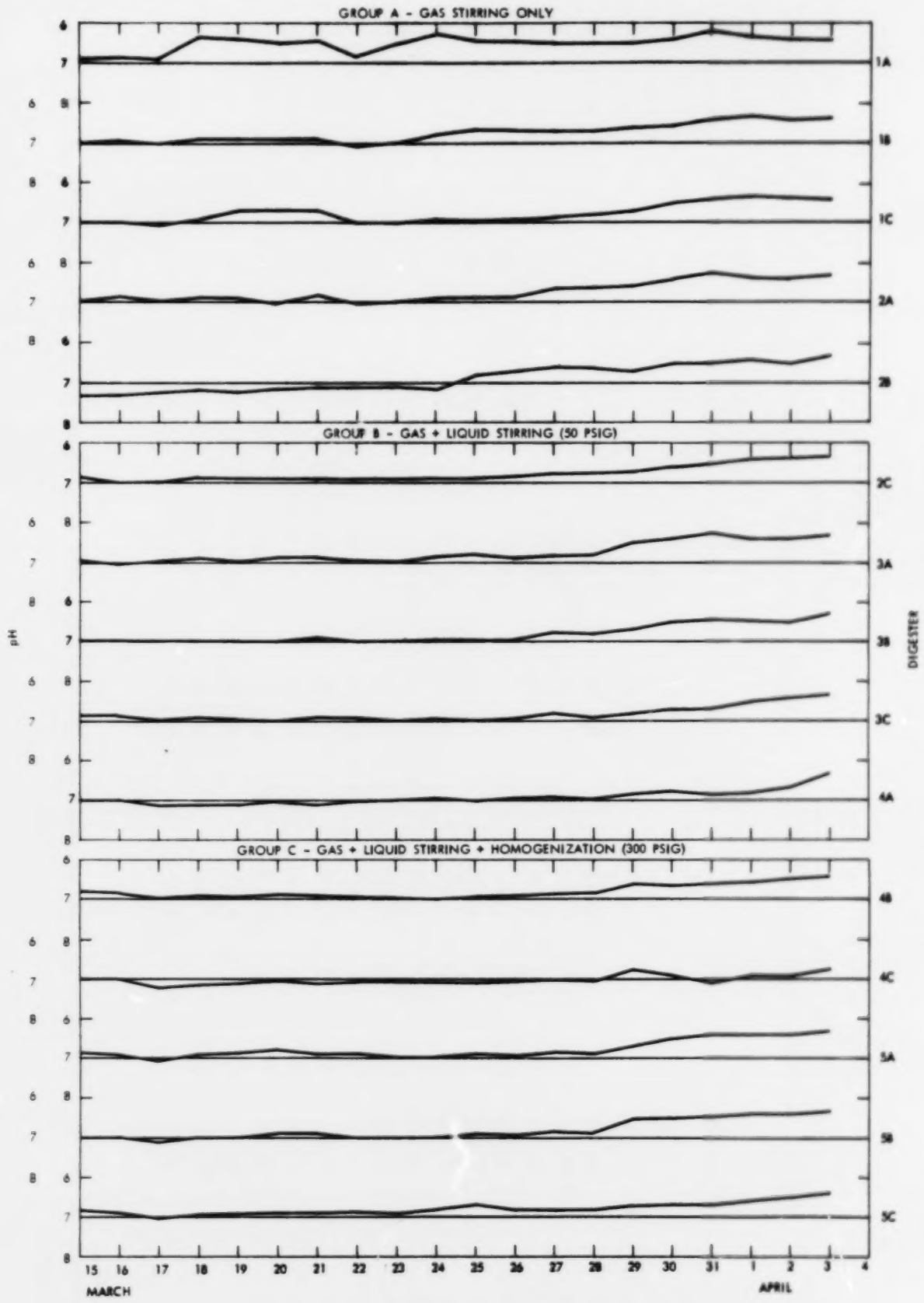

Figure 5 - Variation of pH in Individual Digesters During Experiment V

EXPERIMENT VI

THE EFFECT OF MOTOR OIL ON THE DIGESTION OF PURINA DOG CHOW AND COTTONSEED OIL

The evaluation of the effect of a non-biodegradable lipid on the digestion process was carried out with the digesters filled with 2,160 ml of mixed liquor. The detention time was 21.6 days. There were some difficulties with the break-in period and the Group C digesters were not yet in full balance when the experiment began. The loading protocol is shown on Figure 6 along with the performance data. It is apparent that motor oil had little effect on the digestion of the dog food and cottonseed oil, as neither the gas production rate, the methane content of the gas, nor the pH was affected by its addition.

Since the poorer performance of Group C digesters could have been due to the action of the homogenizers, the liquid pumps were turned off for three days to evaluate this idea. Gas-volume data from each of the three groups of five digesters are averaged and presented below as ratios of the averages with Group A averages the common denominators for all three groups of ratios.

Condition	Group A	Group B	Group C
1	1.00	1.06	0.68
2	1.00	0.99	0.81
3	1.00	1.00	0.89
4	1.00	0.97	0.70

where Group A = gas recirculation only.
B = gas + liquid recirculation (50 psig).
C = gas + liquid recirculation + homogenization (300 psig).

Condition 1 existed on the fifth day (5-4-69) after operation at a daily feed rate of 9 g of dog food and 1 ml of cottonseed oil with both liquid and gas pumps operating.

Condition 2 existed on the fourth day (5-9-69) after 1/2 ml of motor oil was fed daily in addition to the dog food and cottonseed oil. All pumps were operating.

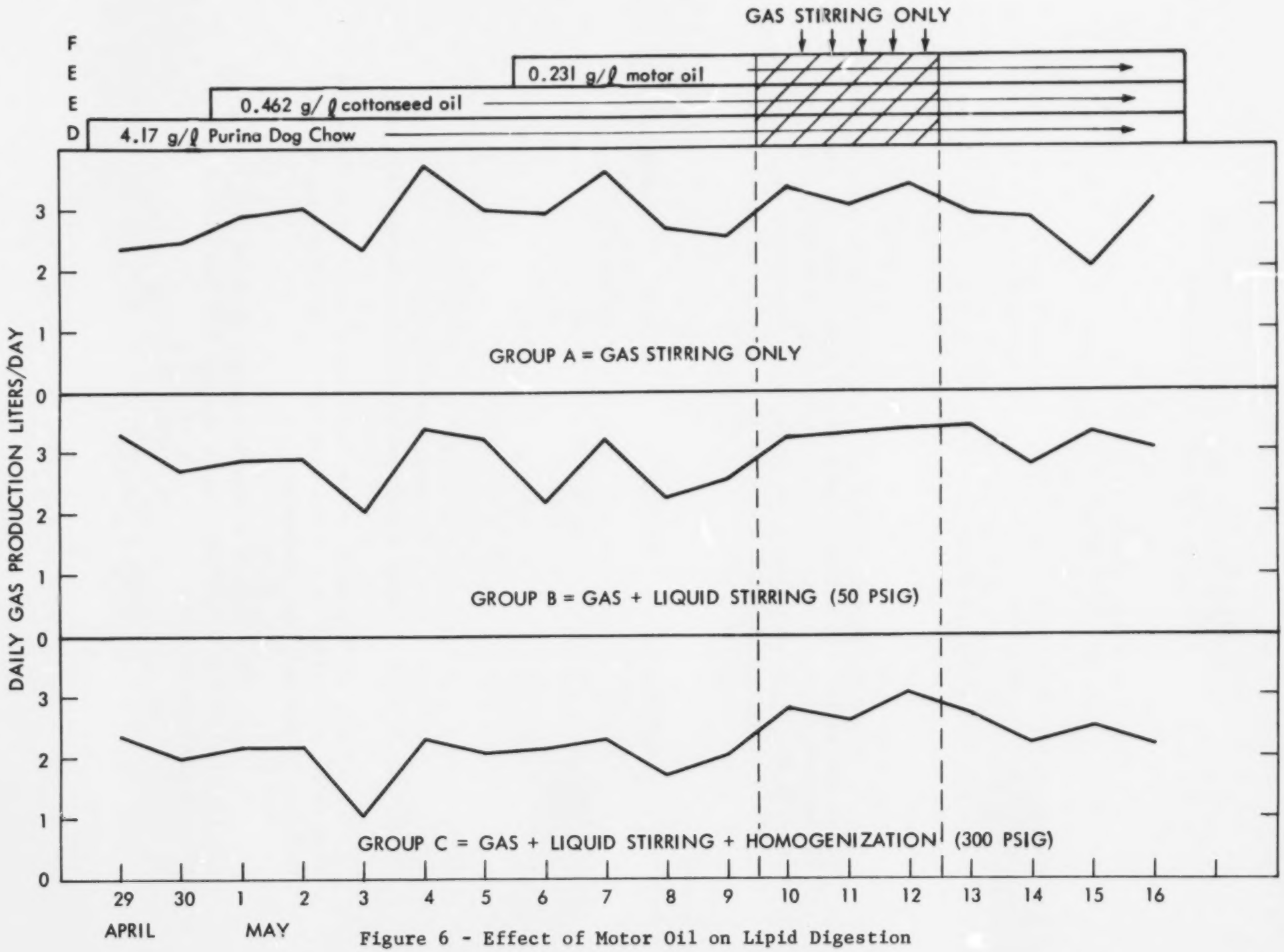
GAS STIRRING ONLY
F
E
E
D
0.231 g/ℓ motor oil
0.462 g/ℓ cottonseed oil
4.17 g/ℓ Purina Dog Chow
DAILY GAS PRODUCTION LITERS/DAY
GROUP A = GAS STIRRING ONLY
GROUP B = GAS + LIQUID STIRRING (50 PSIG)
GROUP C = GAS + LIQUID STIRRING + HOMOGENIZATION (300 PSIG)
29 30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
APRIL
MAY

Figure 6 - Effect of Motor Oil on Lipid Digestion

Condition 3 existed at the end of three days (5-12-69) with only the gas pumps operating. The feed rate was the same as in condition 2.

Condition 4 existed after the liquid recirculation pumps had been turned back on and had been operating for four more days (5-16-69). The feed rate was the same as in condition 2.

The results of the above analysis seem to again implicate high shear homogenization as a negative factor in lipid digestion.

DISCUSSION

The Effect of Shear on the Digestion of Lipids

The results of the experimental program described in this report present a strong caution against the use of high-shear homogenization as a remedy for scum layer formation in full-scale anaerobic digestion. Experiment III produced data to show that lipid digestion in lime-buffered digesters can be slowed by a low-shear homogenization, while lipid digestion is upset by high-shear homogenization equivalent to that produced by a Waring Blendor. Experiments IV and V present data that show a slight process improvement due to high-shear homogenization when soda ash is used as a buffer in a system heavily loaded with lipid. This reversal, however, occurs when the digesters were malfunctioning for another reason.

The anaerobic digestion process like all biological processes is described by the term "steady-state dynamics." The hydraulic analogy is a watershed in which flow into a portion of the system is matched by flow out. If flows are not matched, the result is either flooding or draining of an impoundment. A simplified description of the digestion process is

SLUDGE

(Insoluble Organic Matter)

R_1 ↓ Extracellular Enzymes

(Soluble Organic Matter)

R_2 ↓ Acid Bacteria

Volatile Acids

R_3 ↓ Methanogenic Bacteria

$CH_4 + CO_2$

In a well-operating digester, the rates of reaction in units of material altered per unit time must be equal, $R_1 = R_2 = R_3$. The ability of the organisms to multiply and adapt to increased food levels, however, varies greatly, with $\frac{dR_1}{dt} \neq \frac{dR_2}{dt} \neq \frac{dR_3}{dt}$. All of the technical data available

indicate that $\frac{dR_2}{dt} > \frac{dR_3}{dt}$. It appears that in the case of grease, $\frac{dR_1}{dt} > \frac{dR_2}{dt} > \frac{dR_3}{dt}$ when adequate surface is created between the grease and the digester liquor. The soluble organic matter, in this case fatty acid salts of calcium or sodium or soaps, can have two modes of actions. It can accelerate R_2 so that the volatile acids build up, lower the pH, and inhibit methane fermentation. The result is a "stuck" digester. The other action of the soaps is to directly inhibit methane fermentation by affecting the bacterial surface. This, too, would lead to a "stuck" digester.

Soda ash, which supplanted lime as a buffer for the purpose of reducing foaming, proved harmful because it, also, accelerated hydrolysis too much. The data are consistent with, but do not prove, the following hypothesis: Sodium soaps (sodium salts of long chain fatty acids) would inhibit methane fermentation. Sodium soaps are much more soluble than calcium soaps, but they are still very sparingly soluble. When sodium soaps reach a certain level of concentration in water, the soaps form emulsions. Most of the soap is present as the oil phase of an emulsion, a portion acting as its own emulsifying agent. High-shear now functions to provide interface between the soap micelle and bacteria that degrade the soap to simpler metabolites that can be fermented to methane. McCarty[12] has suggested the use of calcium chloride to reverse sodium oleate poisoning in the same series of articles that advocate the use of sodium bicarbonate.

The results of the study, nonetheless, should not be interpreted as condemning the incorporation of surface mixers in the design of digestion tanks. There is a report[13] of the installation of four 10 hp Lightning mixers on a 100,000 ft^3 tank in the town of Tonawanda, New York, with the very rewarding result of eliminating scum formation. Since most of the scum from the settling tanks is burned, this tank is not subjected to a heavy grease load. The results of this research led to the prediction that the operator of the Tonawanda Plant would be in serious trouble should the situation arise when the grease burners are inoperative at the same time a heavy slug of grease is fed to the digesters as a shock load. While mechanical mixing in addition to that caused by rising gas bubbles is essential for high-rate digester performance, the hydrolysis of heavy grease loads could proceed too rapidly as a result of creating grease-digester liquor interface. In such a situation there would be a build-up of soaps, either calcium soaps from lime, or sodium soaps from soda ash, that would affect the methane bacteria and cause digester upset. Foam production, which is especially severe when lime is used as a buffering agent, may also negate any process advantage due to high-shear mixing.

Ability of Digesters to Degrade Heavy Loads of Grease

It is reported by Basu[14] that heavy grease loads cannot be digested. In Experiment III, during the last three days of fermentation, the Group A digesters were loaded with a feed stock that was at least 43% fat by weight and 70% fat on the basis of COD. This feed produced the most rapid gasification and the highest methane content gas of any sample of feed stock used in the study. High shear that was equivalent to that of a Waring Blendor eventually caused a matched group of digesters, Group C, to go sour. Even the lower level emulsification of the Group B digesters caused malfunction. The level of gas stirring in Group A, however, was such that about two volumes of gas equal to the liquid volume in the digester were pumped to the bottom of the digester each hour. This type of stirring cannot be quantitated, but the digester contents were undergoing a lively bubbling due to gas recirculation.

Optimum Operating Conditions for Sludge Digesters

Loading rate: Manual of Practice 16[15] differentiates "high rate" as opposed to "standard rate" digestion on the basis of loading, 0.15 to 0.40 lb volatile solids/ft^3/day compared to 0.04 to 0.10 lb. volatile solids/ft^3/day. The article goes on, however, to consider mixing as an essential feature of the high rate system. In this study, the digesters were able to accommodate 0.29 lb volatile solids/ft^3/day of the highly putrescible dog food. Since the experimental design was such that most of the time constant feed levels were maintained, not much kinetic data can be derived from the experiments to establish a digestion rate. Still, during the periods of startup, the digesters responded in one day to any load increase that was not an overload. The overload rate was not reached by slow buildup at any time, so a maximum load cannot be set for the experimental system either with a dog food or a combination dog food and cottonseed oil feed.

Temperature: The extreme temperature sensitivity of the process can be seen in Figure 1. The often-published[16] diagram of performance vs temperature of the digester places the optimum temperature of the mesophilic bacteria at 38°C. Occasional shifts in temperature in the digesters used in this study showed that activity increased with temperature from 34° to 41°C. In contrast to our observations, Lyman, McDonnell, and Krup[17] cite experience with full-scale digesters where a 5°C variation had no effect on gas production.

pH: The normal process range recommended for pH is 6.80 to 7.20. The results of the pH measurements in this study imply that the range may be much more narrow, as any drop below pH 7.00 seemed to portend process difficulties.

Microbial ecology: Several hundred microbial smears were examined with the view to characterizing the dominant organisms in the digestion process. No pattern developed that could be discerned by the simple test except that the tetrad packets of cocci reported by Cookson[18] appear to increase during cottonseed oil feeding.

Buffering agent: This research program has also produced results that show that soda ash and lime cannot be used interchangeably as buffering agents. Unless overmixed, lime buffered digesters can tolerate heavy loads of biodegradable oil, while under the same conditions soda-ash buffered digesters go sour. Filbert[19] has analyzed digester startup problems. He quotes both McCarty and McKinney as advocating the use of sodium bicarbonate as a buffer. Soda ash at a pH below 8.3 would be converted from sodium carbonate to sodium bicarbonate.

Statistical Basis for Plant Design

This research program is innovative in the use of replicate digesters and elementary statistical techniques for evaluation of the data. Few other recently published research papers report the use of more than a single digester to establish the design principles and parameters that are used for the construction of full-scale plants. The study of the effects of radioactivity on digestion by Grune, Church, and Kaplan[20] used statistical analysis to evaluate data from a bank of 18 digesters, but the data from that study have little value for design. While the overall research program was conducted with impressive precision, many individual data varied widely from the mean. A design parameter without a measure of variability is valueless. With this in mind, the difficulties encountered in practice with anaerobic digestion are easily understood.

CONCLUSIONS AND RECOMMENDATIONS

The program described in this report was initiated because sound biochemical rationale predicted that the process of digesting waste fats and oils would be accelerated by creating more favorable conditions for bacterial hydrolysis of these water-insoluble materials. The results of the study have not precluded the use of surface mixers or other devices to incorporate scum layers into the main mass of digesting sludge. What has been shown, however, is that if such a process modification is effected, the intensity of the turbulence must be adjusted to the nature of the feedstock.

All waste treatment processes must be highly adaptable to the variable quantity and composition of the feedstock. The anaerobic digestion process is particularly susceptible to upset when any of the process variables are altered. There is no question that the process is far from a state of maximum optimization. There is, however, the question of the possibility of further optimization while retaining the necessary degree of adaptability to feedstock. Real time feedback control techniques that have been adaptable to other chemical processes cannot be used for digestion. Few such techniques are available for characterization of the feedstock and we do not yet fully understand the kinetics of methane fermentation.

Current ideas in sanitary engineering design are that the disadvantages of digestion outweigh the advantages. (See Table I.) Still the majority of sludge in this country is probably stabilized by anaerobic digestion and operators of these digesters are continually faced with the problems of grease overload and scum layer formation.

A continuation of the research program described in this report would surely yield better design parameters for digester mixing. Such a program should include laboratory studies with replicate 1-gal. digesters. Since gas stirring of the type employed in the present study creates a fair degree of turbulence, the baseline process should be digesters that are stirred only by the effervesce of the gas or briefly stirred before sampling. With the quiescent digesters, the effect of increased turbulence on the performance of the digesters in terms of conversion of volatile solids to gas, and stability to shock loads of grease would be evaluated. Natural municipal sludge would be used as a feedstock with authentic sewage grease used as the lipid for shock loading.

Simultaneously with a laboratory program, operators in a selected region, possibly the Missouri River Basin District (because of its many animal-processing facilities), should be asked about their experiences with malfunction due to grease overload.

Information from these two programs will provide design criteria for mixers that will help cure "scum" layers, and can be operated in a manner that will not cause upset by a too rapid saponification of a shock load of triglyceride.

APPENDIX A

APPARATUS AND METHODS

APPARATUS

A schematic drawing of a single digestion unit is shown in Figure 7, while Figure 8 shows the actual components. A later modification was the addition of a 1-ℓ foam trap between the digester and the gas pump. Fifteen such digesters were assembled into a unit that included a circulating water bath. The bath was maintained at 36°C ± 1° during most of the experimental period. The complete assembly is shown in Figure 9.

The most important single aspect of the project was the homogenization units because the entire program has been based on the premise that scum-layer digestion might be accelerated as the material is homogenized into the main body of the aqueous fermenting mass. Homogenization was to be effected by pumping the lipid and aqueous phases together through an orifice under pressure.

A Hoke No. 6528L4B pressure relief valve when fed with a Milton Roy mRoy-110-A diaphragm pump has been shown to incorporate a considerable amount of vegetable oil into water and produces a satisfactory emulsion when the valve is set at less than 500 lb. This finding overcame the greatest potential engineering bottleneck in the project.

A Hoke pressure relief valve was constructed of brass and Teflon. Although the brass was a possible source of copper contamination which would have inhibited bacterial growth, this deleterious effect was eliminated by maintaining an adequate level of sulfide ion in the digester liquor as proposed by Lawrence and McCarty.[20] Another potential problem was a continual change in orifice geometry, due to normal wear or abrasion by grit. A four-day trial with an oil and water system showed that no unusual wear damage to the orifice occurred under these conditions. When the system was used on a sludge that had not been settled, some abrasion of the valve orifice was observed. It was necessary to replace these relief valves several times during the study.

It was not possible to measure the homogenizing pressure with a gauge while the system was in operation. The hydraulic "hammer" from the diaphragm pump would have rapidly destroyed a gauge. An auxiliary hydraulic system with a pressure ballast tank and a snubber on the test gauge was used to set and periodically check pump and relief valve pressures.

Gas recirculation was effected with a small Presso-Vac pump in which the inlet stream was reduced by means of an orifice which consists of glass capillary tubing in which was inserted wire. Frequent cleaning and replacement of the diaphragm and Viton valves were necessary for continuous operation.

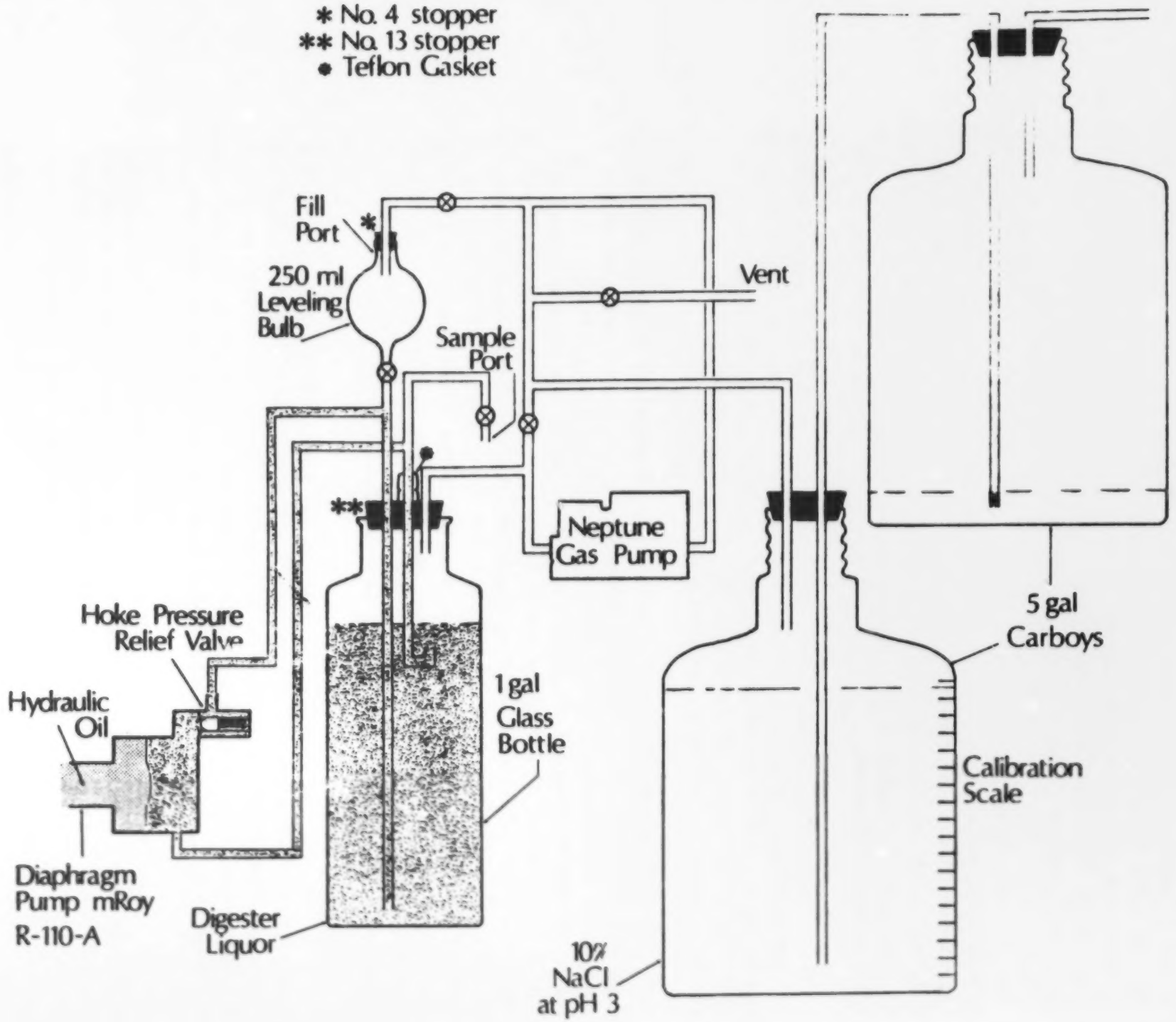

Figure 7 - Schematic Diagram of Digestion Apparatus

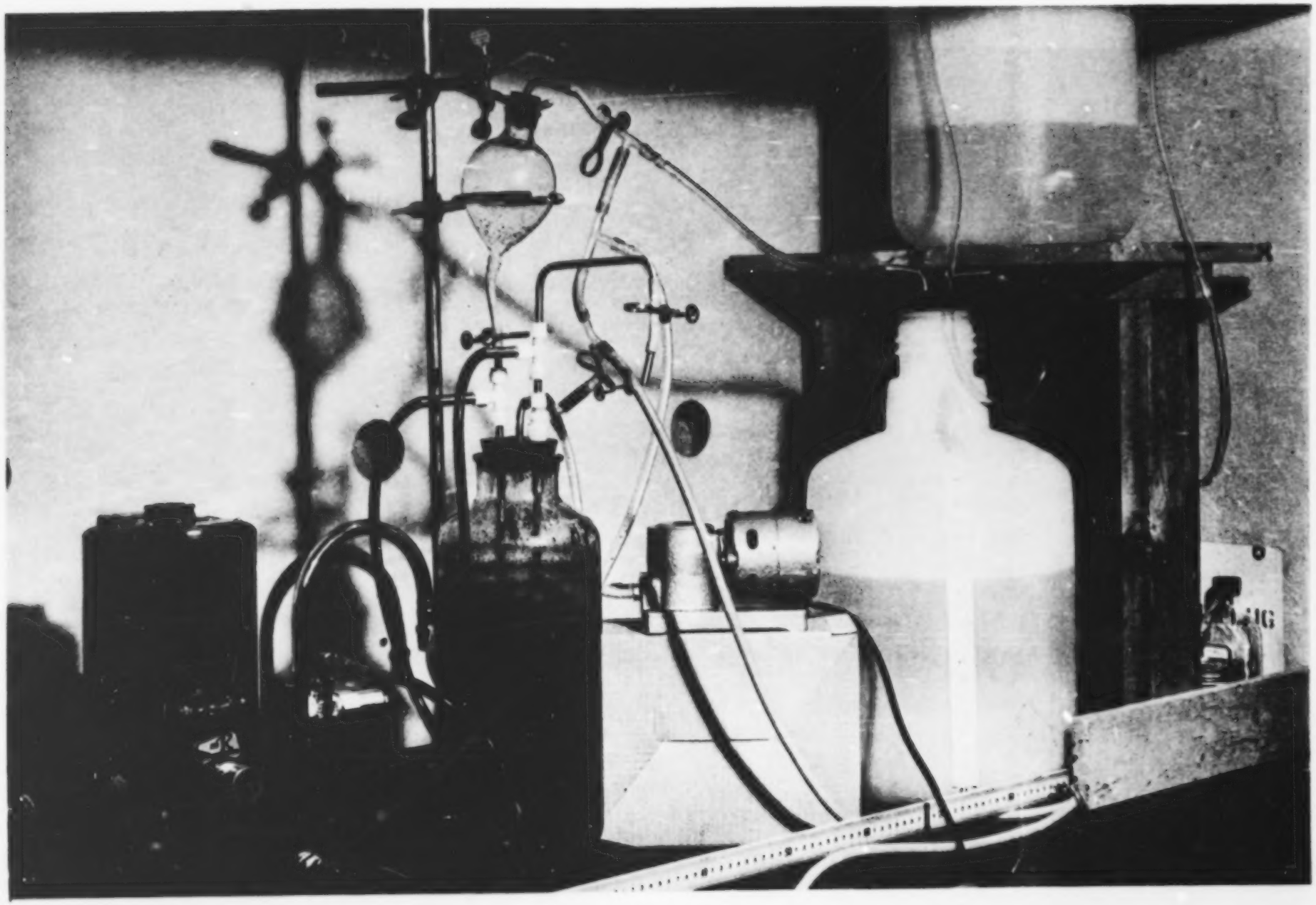

Figure 8 - Photograph of Digestion Apparatus

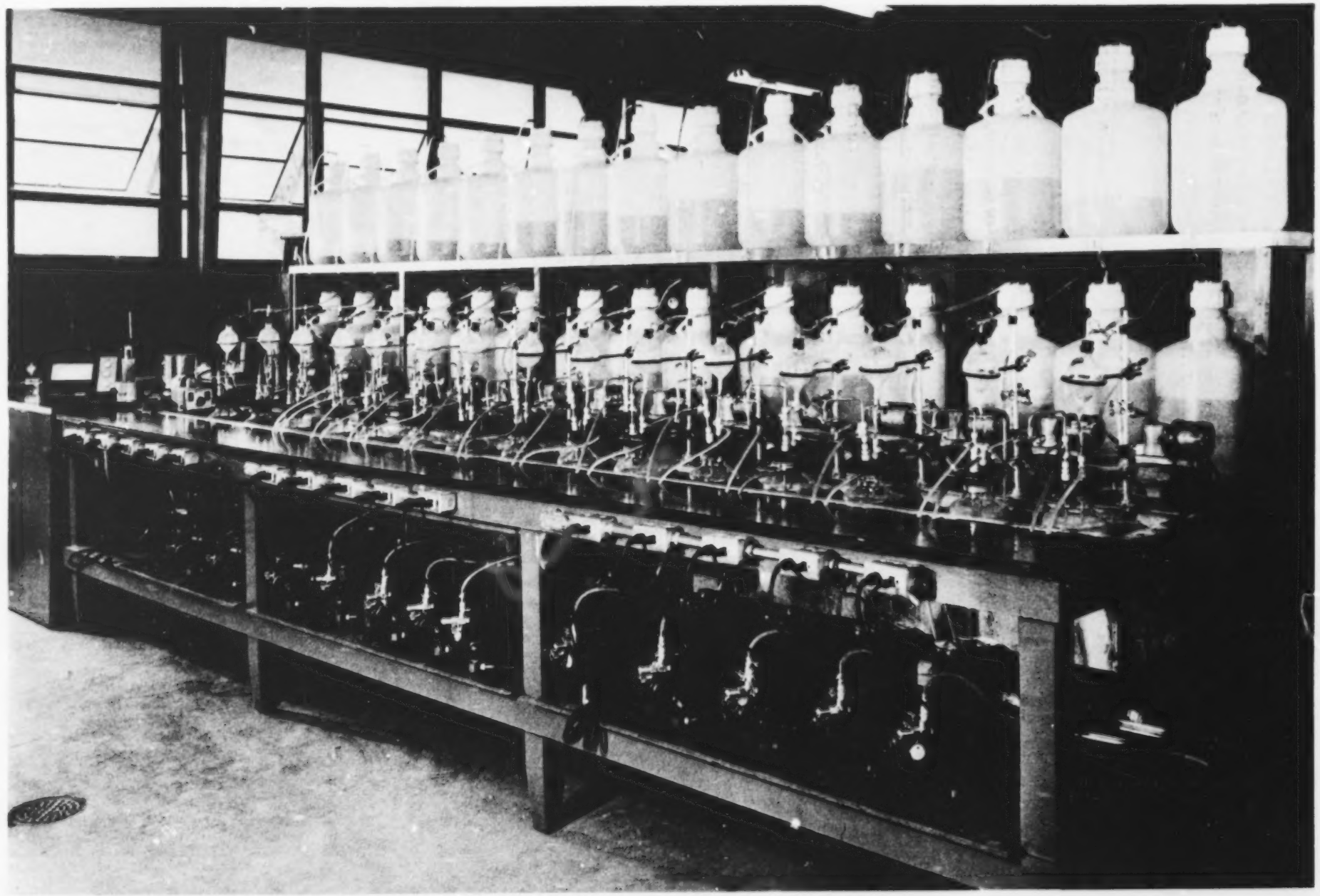

Figure 9 - Fifteen Digester Unit Assembly

Gas was collected by displacement of an acidified 10% NaCl solution from one polyethylene carboy into another carboy which was under a slight pressure. The larger the volume of gas produced, the larger was the pressure on that gas due to hydraulic head. Appropriate conversion factors for pressure and temperatures were used in calculating the amount of gas that was produced.

Gas samples were collected from the system by inserting a glass, gas-collection bulb in the vent line.

Liquid samples were obtained from the pressurized system by opening the sample port. Stainless-steel tubing was used to connect the liquid and gas systems to the glass bottle which served as the digester. Nylon Swagelok fittings were employed where necessary. The liquid exhaust tube was 1/4 in. O.D. which passed through a 3/8 in. O.D. tube placed in the stopper. A Nylon Swagelok 3/8 in. to 1/4 in. union has been bored through, and the 1/4 in. end ferrules were replaced with Teflon ferrules. In this manner a gas-tight seal was maintained while the exhaust tube could be placed to skim the surface or sample the main body of liquid.

Material was introduced into the digester through the liquid and gas return line. Thus, there was no holdup.

ANALYTICAL METHODS

CONVERSION OF DIGESTER GAS VOLUME DATA TO STANDARD CONDITIONS

Several variables were considered in the conversion of the daily gas readings to standard conditions. These are:

1. The time span over which the volume change was observed,
2. Reservoir temperature,
3. Barometric pressure,
4. Head of liquid in the reservoir,
5. Variation in diameter among the reservoirs, and
6. Vapor pressure of the brine solution.

Gas was collected in a polyethylene bottle that was filled with a 10% brine solution acidified to pH 3. Liquid in this reservoir was displaced by the gas produced during digestion into another bottle that was placed superior to the first, as shown in Figure 10. Thus, the gas in the reservoir was under a slight pressure at all times. The datum that was recorded by the operator was the volume of liquid remaining in the lower reservoir. Since digester gas could be vented or CO_2 added during the liquid sampling and feeding operations, this datum was recorded both at the beginning of the day, and after all the daily operations were completed. An adjustment for this variable time period had to be made. The equation for calculating this adjustment is:

$$V_a = \frac{24}{t} (V_1 - V_2) \qquad (1)$$

where V_a = gas produced during elapsed 24 hr

V_1 = previous afternoon reading of liquid content of the lower reservoir in liters

V_2 = morning reading

t = elapsed time in hours between V_1 and V_2.

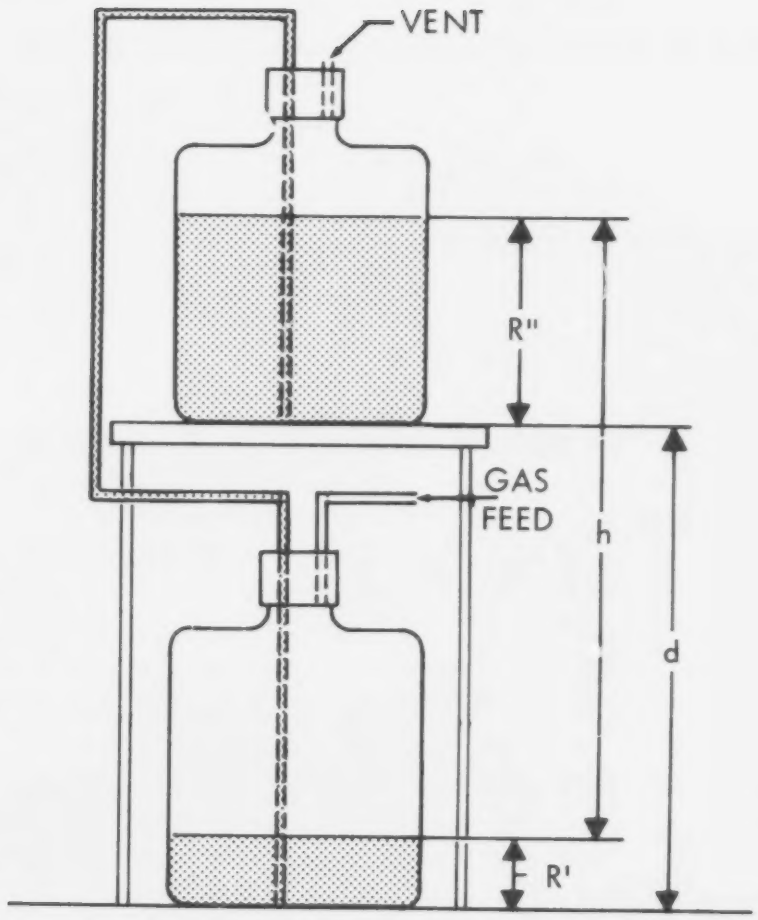

Figure 10 - Diagram of Location of Gas Reservoirs

The equation for calculating the adjustment for reservoir temperature is:

$$V_b = V_a \times \frac{273.2}{273.2 + T} \quad , \tag{2}$$

where T = temperature in °C, and V_b is temperature and time corrected gas volume.

If a table of adjustment factors is calculated, Eq. (2) can be simplified to

$$V_b = V_a \times F \tag{3}$$

A set of factors is shown in Table X.

TABLE X

FACTORS FOR TEMPERATURE ADJUSTMENT OF GAS VOLUMES

Temperature °C	Temperature °F	Factor
20	68	0.932
21	70	0.929
22	72	0.926
23	73	0.922
24	75	0.919
25	77	0.916
26	79	0.913
27	81	0.910
28	82	0.907
29	84	0.904
30	86	0.901

The adjustment for pressure must recognize the barometric pressure, the liquid pressure head, and the vapor pressure of the brine solution in the reservoirs. The equation for this adjustment is:

$$V_c = V_b \times \frac{(B + H - W)}{760} \tag{4}$$

where V_c = gas volume at STP,

B = barometric reading,

H = liquid head,

W = vapor pressure,

each as millimeters of mercury.

In Figure 10,

h = height of liquid exerting pressure,

d = 635 mm, the distance one reservoir was mounted above the other,

and

R' and R" = height of liquid in the respective reservoirs.

These are related as follows:

$$h = d + R'' - R' \tag{5}$$

but

$$R'' + R' = C \tag{6}$$

so

$$h = d + c - 2R' \tag{7}$$

"C" was actually the height of the liquid when it was all in the lower carboy and this amounted to 360 mm. Since the lower carboys were calibrated in liters, the datum that was recorded daily by the operator is liters of liquid. R' is related to V_L (the volume of the lower carboy) by the empirical relationship

$$R' = 18.4\ V_L \tag{8}$$

where R' was in units of millimeters height per liter contained.

The variation in diameter among the 30 carboys that were used as reservoirs did not exceed a maximum of 2% of the average. Since the error in reading the volume of liquid in the carboy exceeded the variation in diameter, the latter variation, while recognized, was not significant.

Since we were interested in the pressure change over the time, t , an average value for R' was obtained by use of the relation,

$$2R' = 18.4\ (V_1 + V_2) \tag{9}$$

where V_1 and V_2 are liquid volumes in the lower carboy at times t , and t_2 , respectively.

To convert the height, H , into pressure head in millimeters of mercury, H , we multipled by the specific gravity of the brine which is 1.07, and divided by the specific gravity of mercury which is 13.8. The correction for change in density due to temperature was too small to consider.

$$H = \frac{1.07h}{13.8} \tag{10}$$

By combining the various expressions, we obtain

$$H = \frac{1.07}{13.8}\left[635 + 360 - 18.4\ (V_1 + V_2)\right] \tag{11}$$

The third pressure correction, the vapor pressure of the brine, is 18 mm Hg at 25°C. The variation of the vapor pressure with temperature in the operating range is too small to be considered. Thus, W = 18 in Eq. (4).

A general expression for standardizing the gas volume can now be obtained by combining Eqs. (1), (3), (4), and (11).

$$V_c = \frac{24F}{t}\left[B + \frac{1.07}{13.8}\left[635 + 360 - 18.4\ (V_1 + V_2)\right] - 18\right]\frac{(V_1 - V_2)}{760} \tag{12}$$

which reduces to

$$V_c = \frac{(V_1 - V_2)\ 0.0316F}{t}\left[59.15 + B - 1.43\ (V_1 + V_2)\right] \tag{13}$$

where V_c is liters of gas at STP that would be produced in a 24-hr period. The actual calculation was performed by an IBM 360 computer.

CHEMICAL OXYGEN DEMAND (COD) DETERMINATIONS

COD determinations were performed as follows:

There was added in sequence to a sample flask, a 1-ml sample of MLSS or MLSS filtrate, 20 ml distilled H_2O, 25 ml of 0.250 N $K_2Cr_2O_7$, 30 ml of H_2SO_4, doped with 22 g silver sulfate, $AgSO_4$, per 9-lb bottle of acid, and 0.4 g $HgSO_4$. The mixture was refluxed for 2 hr and cooled to room temperature. (Cooling is essential if the indicator is not to be oxidized.) Two drops of ferroin indicator were added and the solution titrated to a red-brown end-point with 0.1 N $Fe(NH_4)_2(SO_4)_2$.

Seven replicate COD determinations on a MLSS filtrate gave results with a standard deviation of 4%.

A small portion of the mixed liquor appeared to resist complete digestion. A trace of white oil appears during the digestion which refluxes as a steam distillate. When the mixture is cooled, it solidifies and floats on the top of the aqueous layer.

During the experimental period, COD was determined in both the mixed liquor and the mixed liquor filtrate. Data from the latter determination are presented in Appendix B as soluble COD.

SOLID DETERMINATIONS

The mixed liquor volatile suspended solid (MLVSS) was determined by a slight modification of the Standard Methods[21] procedures. Two changes were made. The usual asbestos pad in the Gooch crucibles was precoated with fine sand (Fisher S-151) and 1 ml of a 2% solution of Rohm and Hass' C-7 cationic polyelectrolyte was added to the 50-ml mixed liquor sample prior to filtration.

Number 3 Gooch crucibles fitted with "F" covers were used. MLSS was determined by heating at 110°C and "Ash" was determined by firing at 600°C in a Blue M-M-25A-1A muffle furnace. In each case, the crucibles were allowed to cool in a desiccator loaded with Drierite. Heating was carried out for at least 16 hr and it was demonstrated that "constant weight" was achieved with both MLSS and ash in this period. Since the amount of inorganic buffer added along with the feed was highly variable, the MLSS and ash are not significant variables. The data from the MLSS-ash are the MLVSS which are presented in Appendix B.

The added polyelectrolyte introduces an error. Since more than 10 mg of polyelectrolyte was needed to produce a satisfactory precipitate (20 mg was used), it was assumed that most of this added material remained absorbed on the MLSS. This would cause a maximum error of the order of + 1.5% with the majority of samples that were examined. Some portion of the polyelectrolyte could also affect the COD determination on the filtrate very slightly.

NEUTRAL LIPID

Neutral lipid was determined by a modification of the method of Loehr and Rohlich.[22] Fifty milliliters of the MLSS was placed in a 250-ml separatory funnel equipped with a Teflon stopcock. One hundred milliliters of acetone was added and the mixture shaken vigorously. Twenty-five milliliters of chloroform was then added and the mixture was shaken again. An additional 75 ml of chloroform was then added and the mixture was shaken gently. Two layers were allowed to separate. The lower layer was removed and filtered through Whatman No. 1 paper. One hundred milliliters of the filtrate was evaporated to a small volume in the hood and this small volume was then transferred to a weighed 50-ml beaker. This smaller beaker was then placed in the oven for at least 16 hr at 110°C before cooling and weighing. Replicate lipid extractions showed excellent precision. Recovery of portions of cottonseed oil added to a composite sludge was 53.5, 55.5, and 56.5% for three determinations. The weight of lipid recovered from the extract was multiplied by 36.5 (20/0.55) to give "LIPID" in grams per liter, which is reported in Appendix B.

METHANE CONTENT OF DIGESTER GAS

The digester gas was analyzed by gas chromatography. A Perkin-Elmer Model 154B instrument equipped with thermister detectors was used. The separation column was packed with 80 to 100 mesh Porapak Q which gave excellent separation of CO_2, CH_4 and N_2. The 4 ft x 0.25 in. column was maintained at 40°C. Helium was the carrier gas. The relative detector sensitivity as determined with analytical grade reagent gases was 1.37 for the CH_4/CO_2 ratio of peak heights.

Figure 11 shows the system that was used to introduce the sample into the instrument. The 250-ml glass gas sampling bulb was equipped with glass stopcocks that were sealed with silicone grease. The Teflon gas sampling valve (large black knob) permitted the evacuation of the sample bulb as well as the stainless-steel sample loop that can be seen behind

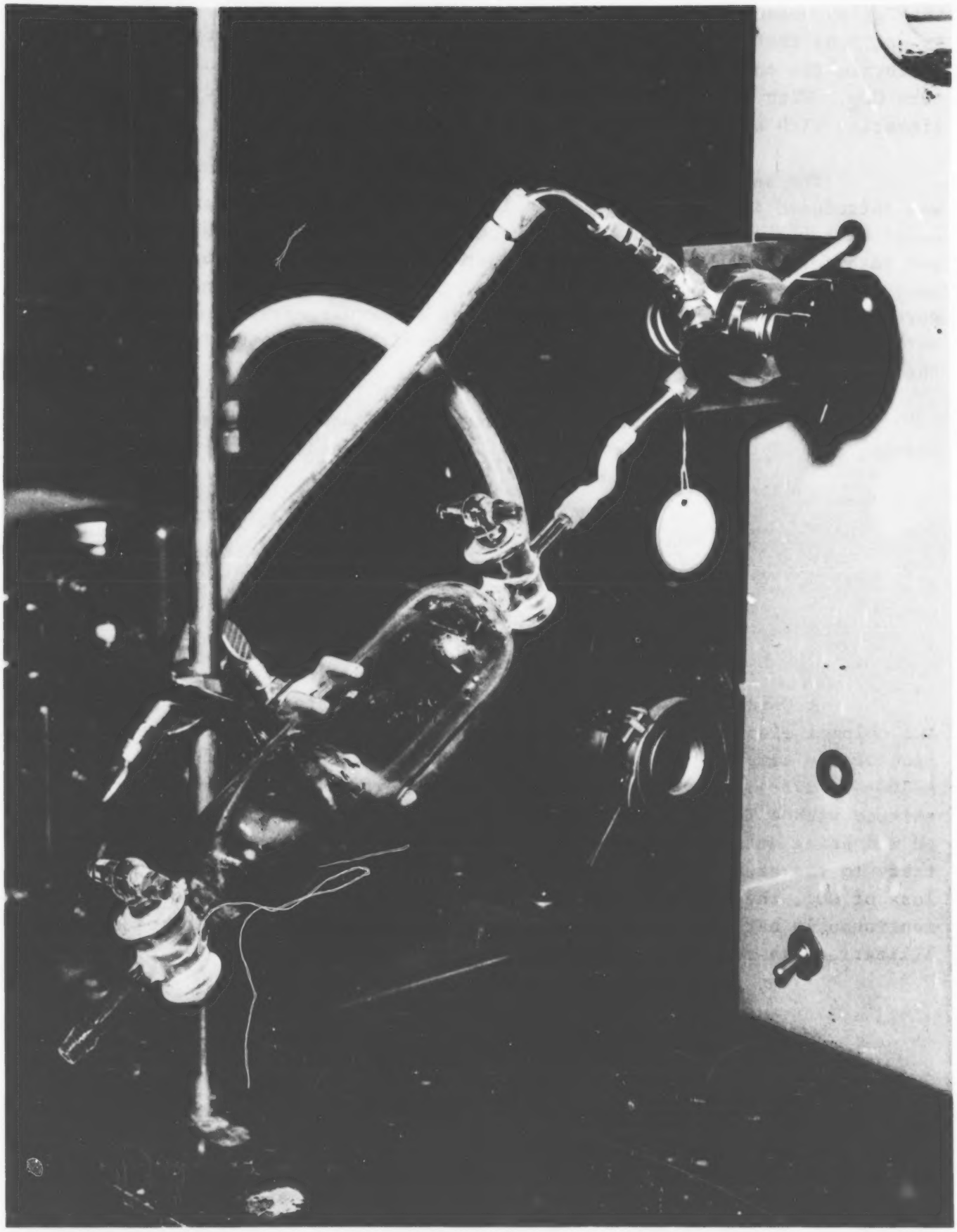

Figure 11 - Sampling Apparatus for Gas Chromatograph

the Teflon valve. A sample loop with a volume of 0.474 ml was used. It is critical that the sample loop not be too large. When we attempted to calibrate the column with a 0.9 ml loop, the column was overloaded with pure CO_2. With the smaller loop, the calibration curve showed complete linearity with all mixtures of CO_2 and CH_4.

The sample system could be completely evacuated. A trace of air was introduced into the sample from the stopcock extension when the evacuated bulb was attached to the gas reservoir exhaust line. This air did not interfere. The column did not separate O_2 and N_2. This was not necessary since, prior to an experiment, the gas reservoirs and digesters were thoroughly flushed with CO_2. The gas reservoir samples were saturated with H_2O which did not produce any response with the fractometer under the prevalent conditions.

Peak height above the baseline was measured on the readout chart for CH_4 and CO_2.

$$\frac{CH_4 \text{ peak} \times 100}{CH_4 \text{ peak} + 1.37\ CO_2 \text{ peak}} = \%\ CH_4$$

HYDROGEN ION CONCENTRATION

A Corning Model-7 pH meter equipped with standard size glass and calomel electrodes was used to determine the hydrogen ion concentration of the sample. The electrodes were calibrated daily with pH 7 buffer. A 100- to 175-ml sample that was freshly withdrawn from the digester was stirred with a Teflon coated magnet that was rotated by a Mag Mix while pH was being determined. The pH varied continuously with time, but contrary to the caution of Standard Methods that the pH would rise due to loss of CO_2, the pH rapidly decreased for a few seconds then decreased continuously but much more slowly. The pH at the apparent point of stabilization was recorded.

MICROBIOLOGY

Samples of the digester contents were taken for microbial assay two to three times each week. These were diluted with one or two volumes of water and spread on glass slides that were fixed by warming. The smears were then stained with Gram's stain and observed with an oil immersion lens at a magnification of 980.

FEEDSTOCK

The digesters were fed Purina Dog Chow that was ground with a Waring Blendor until it passed through a 20-mesh sieve. This material readily wetted and could be transferred with a jet of water. The Purina Dog Chow gave the following analysis:

Carbon	= 48.90%
Hydrogen	= 6.25%
Nitrogen	= 4.49%
Moisture	= 8.52%
Ash	= 8.03%
Lipid	= 7.87%
COD	= 0.943 g/g solid
Maximum Biodegradable	= 84.45%

The cottonseed oil that was used as a typical biodegradable lipid gave the elemental analysis:

Carbon	= 77.71%
Hydrogen	= 11.57%
Nitrogen	= 00.00%
Oxygen	= 10.72% (by difference)

The calculated COD from these results was 2.891 g/g oil. This is slightly higher than the value of 2.785 that can be calculated for pure glycerol trioleate. Based on a density of 0.9140 g/ml at 25°, the oil has a value of 2.64 g COD/ml oil. Thus, in the reported experiments, 0.357 ml of cottonseed oil was the COD equivalent of 1 g of dog food.

The motor oil that was used as a typical non-biodegradable motor oil was Philube Gear Oil - all purpose 80 - MIL-L-2105 which is distributed by Phillips Petroleum. It was not analyzed.

Tapwater originating in the Missouri River was used as liquid makeup.

Sludge specimens were obtained most frequently from the first stage digester of the two-stage digestion system of the southern Johnson County (Kansas) Treatment Plant.

$Ca(OH)_2$, $NaHCO_3$ and Na_2CO_3 were used in various combinations as buffering agents at various time periods during the study. Exact details are given in the sections on Experimental Protocol.

EVALUATION OF HOMOGENIZERS

The combination of diaphragm pump and pressure relief valve was chosen to simulate the performance of the type of homogenizer that is widely used to homogenize milk and a multitude of other polyphasic materials. A typical commercially available homogenizer is manufactured by the Manton-Gaulin Company. The mRoy pump and Hoke pressure relief valve were picked because they were adequate for the job, cheap in relation to available commercial laboratory scale units, and not needed for long-term service.

The homogenizing system was checked for two requirements. First, the homogenizer had to produce a reasonably stable emulsion. A permanent emulsion was not required as the surface of the digester was continually skimmed and any surface lipid film (scum) would be re-emulsified rapidly. The liquid pumps were set to circulate about 3 to 5% of the digester volume per hour. The other requirement was that the shear that produced the emulsion was not so high that it would rupture a significant number of bacteria.

The ability of the system to emulsify cottonseed oil and distilled water was quantified by two rather subjective methods. The emulsions produced by the pump-relief valve system were examined by phase microscopy of a hanging drop. The smaller micelles (globules of oil) could not be counted because of the Brownian movement. Thus the absence of large micelles was the criterion of emulsification. The second criterion was "creaming time," the period required for oil to coalesce and form an observable layer on the surface of the liquid.

Oil:water mixtures, 1:9 and 1:49, were examined in the system with the relief pressure settings of 100, 200, 400, and 800 psig. All pressure settings produced emulsions. This is a relevant fact as the diaphragm pump is equipped with a spring-loaded ball valve that requires 50 psig pressure to open. Therefore, the digesters which were fitted with pumps designed for liquid recirculation only, had some small function as a homogenizer. The pressure setting of 200 to 400 psig produced an emulsion that was comparable to emulsions produced by a Waring Blendor and also by treatment of the mixture with 20 khz sonic energy. The 800 psig setting caused heating of the sample without much observable benefit.

A sample of sludge was circulated through the pump-relief valve system for 10 cycles. The sludge was examined at 970 magnification by phase microscopy and by stained smears. No rupture of cells nor decrease in population could be observed.

APPENDIX B

ANALYTICAL DATA

TABLE XI

RESULTS OF EXTRACTION OF NEUTRAL LIPID
(grams/liter)

Digestor	11-26-68	12-27-68	1-3-69	1-6-69	1-9-69	1-13-69	1-16-69
1A	0.473	0.633	0.844	0.440	1.525	0.917	0.967
1B	0.618	0.720	1.361	0.571	1.252	0.774	0.710
1C	1.345	0.644	0.997	0.760	1.212	0.804	0.775
2A	0.655	0.676	1.205	0.830	1.117	0.808	0.589
2B	0.582	0.898	1.227	1.127	0.957	0.760	0.564
2C	0.655	0.473	1.398	1.062	0.688	0.601	0.382
3A	0.473	--	1.270	1.247	0.495	0.866	0.524
3B	--	0.847	1.514	0.716	0.604	0.389	0.840
3C	0.618	0.916	1.154	0.513	0.950	0.484	0.494
4A	0.473	1.029	1.121	0.422	0.976	0.264	0.884
4B	0.510	1.222	1.121	0.695	0.772	0.204	0.884
4C	0.582	0.804	1.147	0.589	0.491	0.324	1.116
5A	0.364	1.534	1.325	0.640	0.772	0.633	0.909
5B	0.587	0.796	1.412	1.062	0.612	0.316	0.877
5C	--	0.665	0.830	1.069	0.299	0.524	1.040

Digestor	2-10-69	2-13-69	2-17-69	2-20-69	2-24-69	2-27-69
1A	1.274	0.855	1.740	2.311	0.899	0.943
1B	1.110	0.921	1.915	2.799	2.148	1.270
1C	1.198	0.688	2.319	2.097	1.096	0.914
2A	1.106	0.855	2.115	2.592	1.259	0.979
2B	0.890	1.136	2.646	3.437	1.875	1.117
2C	1.085	0.895	1.445	1.860	1.467	1.147
3A	0.688	0.855	1.951	1.219	1.558	1.372
3B	0.990	0.444	2.341	2.282	1.736	1.147
3C	0.579	1.147	1.410	2.584	0.761	0.681
4A	0.717	0.430	0.870	1.168	0.575	0.815
4B	0.968	0.579	1.023	1.303	0.466	0.797
4C	0.874	0.612	1.227	1.598	0.528	0.848
5A	0.535	0.597	1.219	1.765	0.946	0.859
5B	0.568	0.510	1.390	2.821	0.892	0.830
5C	0.491	0.622	1.241	1.700	0.692	1.041

TABLE XI (Concluded)

Digester	3-3-69	3-10-69	3-17-69	3-20-69	3-24-69
1A	0.655	0.345	0.545	1.000	1.310
1B	0.560	0.455	0.724	0.898	1.385
1C	0.542	0.578	0.713	1.062	1.156
2A	0.560	0.695	0.644	1.276	0.884
2B	0.651	0.836	0.112	0.796	1.094
2C	0.778	0.575	0.935	1.047	1.178
3A	0.793	0.691	0.858	1.342	0.935
3B	0.716	0.484	0.705	1.149	0.898
3C	0.331	0.444	0.331	0.404	0.225
4A	0.371	0.444	0.356	0.465	0.422
4B	0.120	0.527	0.585	0.607	0.509
4C	0.360	0.469	0.465	0.531	0.458
5A	0.462	0.433	0.658	0.538	0.589
5B	0.371	0.495	0.422	0.538	0.502
5C	0.469	0.447	0.367	1.058	0.716

Digester	3-27-69	3-31-69	4-3-69	4-7-69*
1A	1.916	2.105	3.618	8.425
1B	1.207	1.960	2.912	10.970
1C	1.604	1.993	2.865	5.600
2A	1.873	1.491	1.923	9.970
2B	1.113	1.356	2.920	11.991
2C	1.545	1.429	2.876	11.450
3A	1.789	0.825	1.734	7.923
3B	1.120	0.978	1.628	5.868
3C	1.124	0.393	0.284	4.945
4A	1.291	0.342	0.756	3.607
4B	1.353	0.469	0.363	6.272
4C	0.855	0.484	0.458	5.770
5A	2.043	1.156	1.289	8.850
5B	1.873	0.680	1.607	7.577
5C	1.887	0.735	0.636	5.530

* Extraction of acidified samples.

TABLE XII

MIXED LIQUOR VOLATILE SUSPENDED SOLIDS
(grams/liter)

Digester	12-3-68	12-10-68	12-30-68	1-7-69	1-14-69	2-18-69	2-25-69
1A	19.5	17.3	12.8	12.8	7.6	--	13.2
1B	--	21.2	12.9	6.2	6.7	--	16.0
1C	--	20.4	13.1	4.6	6.0	16.2	14.2
2A	16.9	19.8	13.0	4.3	--	14.9	15.2
2B	17.7	18.6	12.9	5.6	--	16.8	13.8
2C	19.0	5.3	8.4	3.1	4.4	12.3	14.6
3A	15.9	5.8	12.4	5.9	3.6	8.6	11.2
3B	18.7	5.0	7.6	3.3	7.1	14.2	18.9
3C	20.0	5.2	8.4	3.1	1.1	10.4	8.2
4A	--	4.9	6.6	2.2	1.2	--	16.3
4B	17.4	6.1	8.9	4.1	3.8	12.0	15.7
4C	16.2	9.7	8.0	3.0	4.0	13.0	17.3
5A	14.0	5.7	7.3	4.4	2.4	12.7	19.7
5B	14.8	6.7	8.0	4.3	1.8	12.8	13.3
5C	13.2	12.4	7.4	4.2	1.9	13.8	14.1

Digester	3-4-69	3-11-69	3-18-69	3-25-69	4-1-69
1A	13.9	13.5	22.0	24.2	16.5
1B	14.2	20.1	23.8	24.9	14.5
1C	10.1	18.3	19.4	21.0	18.4
2A	14.6	20.1	20.8	20.7	16.5
2B	14.4	20.0	24.6	24.8	23.0
2C	17.0	17.7	10.6	14.9	11.8
3A	16.1	16.9	18.6	16.8	10.5
3B	15.9	16.6	15.2	16.4	10.2
3C	12.7	16.2	8.9	16.8	14.9
4A	14.5	15.8	17.6	16.2	15.3
4B	5.0	16.9	15.1	16.2	13.4
4C	14.5	13.5	15.4	15.0	14.5
5A	14.0	14.8	17.2	17.4	13.4
5B	14.1	13.8	17.0	17.0	13.0
5C	12.3	14.4	15.4	14.7	13.3

TABLE XIII

SOLUBLE COD
(grams/liter)

Digester	11-26-68	12-3-68	12-10-68	1-7-69	1-14-69	2-18-69	2-25-69
1A	--	15.6	15.8	10.4	6.1	--	18.9
1B	6.9	14.1	15.6	9.9	5.5	--	21.8
1C	8.8	12.8	14.4	11.1	6.0	15.6	20.0
2A	7.4	15.0	17.7	9.7	6.6	20.3	23.1
2B	7.9	14.2	14.8	6.4	5.5	22.3	25.9
2C	8.1	13.7	14.9	12.4	8.3	16.2	19.6
3A	8.0	14.7	15.3	14.9	7.1	29.8	27.0
3B	8.8	12.9	15.0	11.7	10.2	19.3	29.0
3C	8.8	13.5	15.6	11.0	3.8	22.8	31.3
4A	7.3	12.4	11.5	12.2	6.9	--	24.4
4B	10.6	15.5	14.6	13.1	10.6	16.9	23.6
4C	7.9	14.5	19.7	14.8	13.2	16.5	21.9
5A	12.6	13.9	12.3	12.6	10.7	22.6	29.1
5B	8.5	14.0	14.2	13.8	12.3	32.7	31.1
5C	8.7	14.3	17.6	13.7	12.3	3.7	23.1

Digester	3-4-69	3-11-69	3-18-69	3-25-69	4-1-69
1A	16.1	15.9	18.9	26.3	31.0
1B	18.1	19.9	25.5	21.2	22.7
1C	15.5	17.1	20.7	18.7	23.7
2A	15.0	22.2	23.5	19.7	21.2
2B	21.1	21.2	23.4	17.8	22.6
2C	15.5	16.8	21.3	19.6	19.1
3A	22.1	26.0	17.6	17.2	15.2
3B	24.2	26.8	21.2	18.5	16.0
3C	27.4	27.6	22.2	21.5	20.6
4A	16.0	22.1	18.5	17.5	14.3
4B	16.1	22.6	18.0	17.3	17.0
4C	21.0	25.3	17.5	18.6	13.4
5A	18.6	19.6	22.0	21.2	17.5
5B	21.8	17.6	22.0	21.0	14.8
5C	16.0	16.2	18.4	16.8	16.3

TABLE XIV

METHANE CONTENT OF DIGESTER GAS
(percent)

Digester	10-8-68	10-31-68	11-6-68	11-12-68	11-21-68	11-26-68	12-5-68	1-10-69	1-17-69
1A	46	45	57	56	50	55	54	57	64
1B	45	49	55	57	49	56	61	57	63
1C	57	46	55	53	49	53	56	57	62
2A	50	47	54	58	47	54	56	58	63
2B	50	49	45	35	46	55	54	58	65
2C	62	49	57	57	48	55	54	41	52
3A	41	45	53	52	49	55	57	34	48
3B	48	47	56	57	48	55	53	42	50
3C	44	47	53	56	49	54	55	49	52
4A	55	46	54	56	43	54	55	43	52
4B	55	48	55	55	52	55	54	44	46
4C	44	47	55	55	52	55	54	39	38
5A	52	44	53	53	45	50	55	41	41
5B	50	47	55	57	33	52	55	37	42
5C	51	48	55	58	50	53	54	38	34

TABLE XIV (Concluded)

Digester	2-7-69	2-14-69	2-21-69	2-28-69	3-14-69	3-21-69	3-28-69	4-4-69	5-16-69
1A	50		49	56	56	31	38	50	54
1B	42	53	47	57	50	50	52	48	52
1C	51	54	47	56	53	50	52	46	54
2A	50	54	50	62	52	52	53	47	54
2B	50	50	42	44	54	54	51	46	53
2C	50	55	52	58	50	52	58	48	53
3A	41	53	51	58	51	52	53	45	55
3B	58	53	50	57	51	52	53	48	53
3C	43	47	19	34	52	43	52	46	53
4A	53	55	50	51	52	53	55	32	54
4B	52	55	54	61	52	53	54	49	54
4C	50	56	54	9	49	54	55	52	52
5A	50	51	51	56	52	51	53	40	51
5B	44	51	47	26	52	51	53	41	52
5C	46	51	53	59	52	52	53	50	52

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